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Date: April 18th 2001

"... a man's reach should exceed his grasp,
or what's a heaven for?"

(Browning)

"The work of teaching and organizing the others fell naturally upon the pigs, who were generally recognized as being the cleverest of animals"

(George Orwell, Animal Farm, 1945)

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Lactational Muscle Protein Mobilization and Sow Performance** submitted by **Emma Jayne Clowes** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Nutrition and Metabolism**.

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Date: 2001 April 17

DEDICATION

I dedicate this thesis to my Father and brother Richard who have patiently provided support and encouragement throughout my graduate student years, and to my Mother who taught me to smile whatever the problem and who is always with me. Without their support I would not have made it this far.

ABSTRACT

During lactation first-parity sows, under nutritional stress, maintain milk production at the expense of their body reserves (protein and/or fat). However excessive weight loss, and especially muscle protein loss, in lactation is associated with a reduction in litter growth and subsequent reproductive performance (lower ovulation rate and embryo survival). This protects the sow's body reserves, and ensures her survival and the subsequent proliferation of the species, albeit with a potentially smaller subsequent litter size. A critical metabolic issue for the lactating animal is therefore the level of body reserve depletion at which it becomes necessary to reduce the commitment to the current progeny (reduce milk production). A series of experiments were conducted on lactating first-parity sows to determine 1) the level of body reserve depletion at which a reduction in the sow's lactational and reproductive performance occurs, and 2) how the sow's protein metabolism is regulated during lactation. In the first experiment, during very restricted feeding (50% ad libitum) animals maintained milk protein production by a) mobilization of body protein reserves, and b) improving N economy by reducing urinary N excretion. Sows fed by gastrostomy, to 125% of ad libitum intake, directed incremental protein towards maternal reserves rather than to additional milk production. This indicated that lean growth remains a priority in the young reproductive female animal. A second experiment induced lactating sows to lose different amounts of body protein (as opposed to energy) and a small amount of fat. The results of this study suggest that a critical level of protein loss (10 to 12% parturition protein body mass) is associated with a decline in milk production and ovarian function at weaning. In a third experiment, sows

were fed during gestation to achieve a standard or large body mass, and were thereafter fed two levels of protein to induce varying amounts of protein loss (-11 and -17% parturition protein body mass). A larger body mass at parturition delayed attainment of a critical protein loss, and therefore the decline in animal performance. However, regardless of initial body mass muscle protein mobilization per se does not favour optimal milk protein production.

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CHAPTER ONE
REVIEW OF THE LITERATURE

1.1 INTRODUCTION

Lactation is an interesting physiological state in which non-mammary tissues and organs alter their metabolism to divert energy and other substrates to support milk biosynthesis and mammary function. The energy and nitrogen requirements of the lactating sow are more than double that of the dry and/or gestating sow, primarily because the mammary gland starts to synthesize and secrete milk to support growth of the sow's progeny. In so doing, the normally (≥ 85% reproductive cycle) quiescent mammary gland becomes highly metabolically active, and accounts for ~75% of the sow's lactational energy requirement (Aherne and Williams, 1992) and at least 50% of her amino acid requirement. Mammary gland protein synthesis accounts for 32 to 46% of total daily amino acid flux in the lactating goat, depending on milk yield (Champredon et al., 1990; Baracos et al., 1991).

Increased feed intake (hyperphagia) provides the higher energy, protein and other nutrient requirements of lactation to maintain milk production. In the domestic pig however, milk production has increased at least 20 to 30% over the last 10 years (NRC, 1988; 1998), but genetic selection for increased lean growth and improved reproductive traits has tended to reduce sow feed intake (ten Napel et al., 1995; Kerr and Cameron, 1996; Eissen et al., 2000). As a consequence, feed intake in lactation is often not sufficient to provide for the requirements of milk biosynthesis and maintenance of mammary function, making the sow increasingly draw more upon her body reserves (protein and/or fat) to supply the additional energy and nutrients and maintain lactation. Excessive mobilization of maternal body reserves (protein and/or fat) results in a reduction in animal performance in both the current (lactational performance) and subsequent (reproductive performance) reproductive cycle, in favour of maintaining the animal's tissue reserves and promoting her survival. These effects have been reviewed by many researchers (Aherne and Kirkwood, 1985;

Einarsson and Rojkittikhun, 1993; Prunier et al., 1993; Aherne et al. 1995; Foxcroft et al., 1995; Cole and Mullan, 1996).

Inhibition of the current reproductive cycle, due to a reduction in milk production and/or milk protein and fat composition, causes slow progeny growth in lactation. This may or may not delay the time taken by the progeny to reach slaughter weight. Inhibition of the subsequent reproductive cycle is more costly (to the producer) as it involves impairment of the animal's reproductive axis such that commencement of the next reproductive cycle is delayed or even prevented (anestrus). In polytocious species, such as the pig, and in animals that are genetically prone to bearing multiple progeny the reduction in reproductive function may also involve a smaller subsequent litter size, due to a reduced ovulation rate and/or embryo survival.

Mechanisms that inhibit lactational and reproductive performance due to nutritional deficiencies may differ, and neither is clearly understood. Evidence exists that relate the size of the energy deficit in lactation either directly or indirectly to inhibition of animal performance. Low energy intakes in lactation are associated with reduced lactational (Brendemuhl et al., 1989; Tokach et al., 1992a) and subsequent reproductive performance (Elsley et al., 1968; Reese et al., 1982, 1984; Nelssen et al., 1985; Johnston et al., 1989). These effects are likely mediated by dynamic changes in the animal's energy reserves during lactation that are primarily adipose tissue, but proteinaceous tissue such as skeletal muscle may also be involved.

1.1.1 Relationship between an animal's adipose and protein stores and performance

A positive relationship between the size of an animal's body fat stores, feed intake, and reproductive performance has been long acknowledged, and led to the development of the 'lipostat' or 'adipostat' hypothesis (Kennedy, 1953; Frisch and McArthur 1974; Frisch et al., 1980). It was similarly hypothesized that there is a 'threshold' level of body fat below which reproductive failure will occur in the sow (Elsley et al., 1968; Maclean et al., 1969). These hypotheses suggest that circulating concentrations of a hormone secreted by adipose tissue reflect levels of body fatness and acts as a signal to control food intake, energy homeostasis, and also possibly reproduction.

The concept of a circulating satiety factor was strengthened by the discovery of recessive mutations in obese (*ob/ob*) and diabetic (*db/db*) mice that were associated with hyperphagia, decreased energy expenditure, and early onset of obesity (Ingalls et al., 1950). Parabiosis of wild-type mice with *ob/ob* mice, and *db/db* mice with *ob/ob* mice suppressed weight gain in the *ob/ob* mice, and parabiosis of *db/db* mice with wild-type mice caused hypophagia in the lean wild-type mice (Hausberger, 1959; Coleman and Hummel, 1969; Coleman, 1973). These results together suggest that the *ob* locus is necessary for the production of a satiety factor, possibly produced and secreted by adipose tissue, and that the *db* locus encodes a molecule required for response to this factor, such as a receptor.

The *ob* locus has now been cloned (Zhang et al., 1994) and its product, called leptin (derived from the Greek root *leptos* meaning thin), causes reductions in food intake, body weight, and body fat when injected intraperitoneally into either wild-type or *ob/ob* mice (Halaas et al., 1995). Leptin is secreted like a hormone from adipose tissue and negatively feeds back on the brain to control energy homeostasis. Leptin is thought to limit obesity in a normal individual, when nutrients are abundant, and regulate the neuroendocrine system in starvation via negative feedback to brain centres controlling energy homeostasis (Ahima et al., 1996). Many of leptin's effects on food intake and energy expenditure are discussed in Section 1.7.6.

Although there is fairly strong evidence linking leptin to reproductive function in rodents the evidence is not as compelling for other species, including the pig (see Section 1.7.6). This is not surprising, because most complex functions have multiple regulators, so it would be unusual that only one mechanism communicates nutritional status to such an important physiological function as the reproduction. Leptin cannot be discounted as a regulator of the hypothalamic-pituitary-ovarian axis in non-rodent species, but rather other mechanisms such as the availability of metabolic substrates (Wade et al., 1996; Schneider et al., 2000b) are also likely to be involved. Changes in the animal's protein mass may also signal the changes in animal performance observed in lactating sows under poor nutritional conditions. Leptin may also be involved in this mechanism because muscle tissue expresses both leptin mRNA and protein, but a 10-fold lower levels than adipose tissue (Wang et al., 1998). Furthermore, peripheral leptin levels decrease with a reduction in energy status suggesting that leptin could signal changes in the animal's energy status. However, it is not known if leptin levels change with protein deficiency. Whittemore and Morgan (1990) suggested that a sow's lipid mass should be expressed as a function of its

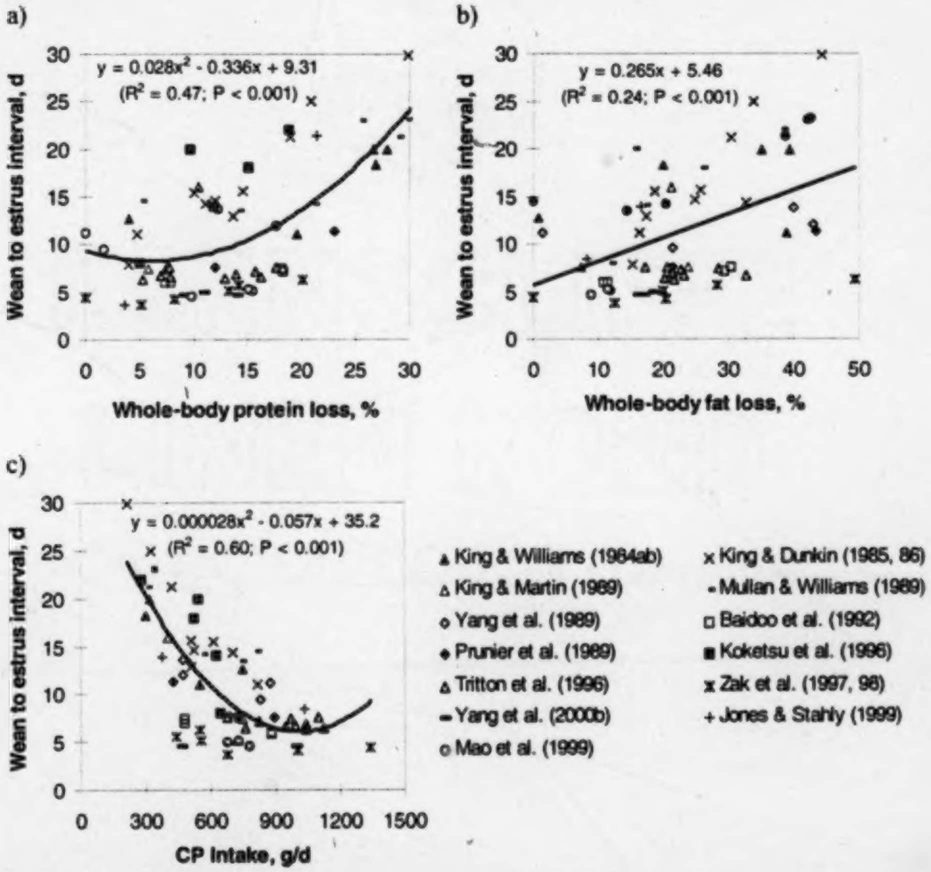

Figure 1-1 Regression analysis of subsequent wean-to-estrus interval against the sow's estimated whole-body a) protein and b) fat loss in lactation, and c) lactational crude protein intake. Whole-body protein and fat mass are presented as % parturition tissue mass, and were estimated from the equations of Whittemore and Yang (1989)

protein mass. If the animal's lipid mass was smaller than its protein mass reproductive efficiency would be compromised (Whittemore et al., 1988; Yang et al., 1989). No account is taken by this hypothesis of the fact that a large decrease in protein mass could dramatically reduce an animal's performance without reducing her protein to fat ratio below unity. The mechanisms behind this hypothesis are unknown.

Regression analysis of data from 16 experiments from the literature indicate that loss of the sow's calculated whole-body protein mass during lactation accounts for almost half the variation in the sow's post-weaning reproductive performance (wean to estrus interval) (Figure 1-1a). Whereas, less than a quarter of the variation in the same data set was accounted for by loss of body fat mass (Figure 1-1b). This data set also provides evidence for a 'critical amount' of protein loss associated with inhibition of the subsequent reproductive cycle (Figure 1-1a), which appears to be loss of between 10 and 15% of the sow's initial (parturition) protein mass. Similarly, the sow's post-weaning reproductive performance appears to decline upon feeding less than 500 to 600 g CP/d in lactation (Figure 1-1c). Protein restriction in lactation also negatively impacts on other reproductive variables such as LH pulsatility and follicular dynamics post-weaning (see Section 1.7.4).

Low protein intakes in lactation are also associated with reduced litter growth rates, milk production (Kusina et al., 1999b), and changes in milk composition (King et al., 1993ab; Kusina et al., 1999b). Similar reductions in lactational performance due to inadequate dietary protein intake have been observed in other species including the dairy cow (Botts et al., 1977) and rat (Pine et al., 1994abc). These effects of inadequate protein intake are likely due to excessive loss of body protein reserves. Thus, changes in the sow's body protein reserves, and in particular muscle mass (predominant source of mobilizable protein, see Section 1.2), and the signals emanating from such changes are likely involved in inhibition of both lactational and reproductive performance. This review of the literature will focus on a number of topics, and these include:

- Muscle protein mobilization and the mechanisms involved in this mobilization.
- Various methodologies for measuring changes in muscle protein mobilization.

- How nutrition in gestation and lactation impacts on the sow's fetal and mammary gland growth and development, lactational performance, and reproductive performance.
- How maternal protein loss in lactation impacts on the lactational and reproductive performance of a sow.

1.2 Skeletal muscle protein mobilization

Skeletal muscle protein constitutes approximately 45% of the total protein in the adult body (Young, 1970), and is the main reserve of body protein accessible for mobilization during nutritional, physiological and pathological states where protein requirements exceed the dietary supply (Allison et al., 1963; Allison and Wannemacher, 1965; Swick and Benevenga, 1977). This tissue is divided into three types that contain:

1) predominantly red, oxidative, slow-twitch (Type I) fibres e.g. diaphragm and postural muscles such as the soleus.
2) predominantly white, glycolytic, fast-twitch (Type IIB) fibres e.g. tensor fascia latae
3) mixed-fibre type muscles which contain both red, oxidative (Type I), intermediate (Type IID), and white glycolytic (Type IIB) fibres e.g. gastocnemius, longissimus, extensor digitorum longus, and triceps muscle.

The mixed-fibre type muscle comprises the majority of muscle in the body (Ariano et al., 1973; Maltin et al. 1989; Delp and Duan, 1996), and is the main source of mobilizable protein in times of nutrition stress (Goodman and Ruderman, 1980; Baillie and Garlick, 1991; Fang et al., 1998). In muscle undergoing protein mobilization, the glycolytic muscle fibres appear to be preferentially targeted for degradation (Henriksson, 1990). Other conditions, such as exercise, increase the proportion of oxidative and intermediate fibres, and decrease the proportion of fast-twitch glycolytic fibres in this muscle (McAllister et al., 1997).

Myofibrillar protein is the most abundant (50 to 60%) protein in skeletal muscle (Helander, 1961), and is the main protein mobilized in skeletal muscle in times of nutritional stress, such as starvation (Li and Wassner et al., 1984; Lowell et al., 1986a). Myofibrillar protein is composed of the normally long-lived proteins actin and myosin (half-life 15 to 30 d; Bates and Millward, 1983; Bates et al., 1983). Sarcoplasmic and membrane proteins make up the remainder of skeletal muscle proteins, and these proteins have half-lives of minutes and hours rather than days. The functions of myofibrillar proteins include: maintenance of cell integrity, contractile function, a source of gluconeogenic carbon, and a potential source of essential amino acids. The latter two functions of myofibrillar protein, which are enabled by muscle protein mobilization, will be discussed in this review.

Net protein mobilization occurs when the rate of protein breakdown exceeds the rate of protein synthesis. Both these processes must be measured in order to understand the degree of protein mobilization occurring and the mechanisms involved. Various direct and indirect methods for measuring these processes are described in Section 1.3. There is a paucity of information regarding skeletal muscle protein turnover in lactating animals, and especially in large domestic species, such as the pig. Therefore the effects of nutritional stress on muscle protein synthesis and degradation reviewed here will be predominantly based on rodents in either a basal (fed or non-atrophying) or activated (starved or denervated) state. Wherever possible, relevant work in lactating animals will be included.

1.2.1 Protein synthesis in skeletal muscle

Many physiological states, including aging and starvation, reduce the rate of muscle protein synthesis (Millward et al., 1976); starvation (4 d) reduced total protein synthesis in mixed-fibre muscles in 100 g, 200 g (8 week old), and 500 g (16 wk old) rats by 70, 50, and 25%, respectively. The rate of muscle protein synthesis was also reduced in septic rats (Fang et al., 2000). Starvation decreased the rate of myofibrillar protein synthesis by 50%, but only decreased the rate of protein synthesis in sarcoplasmic protein by 30% in the adult rat (Bates and Millward, 1983).

Muscle protein synthesis has been studied in a both rodents and ruminant species in lactation. In the ruminant (Vincent and Lindsay, 1985) and mouse (Millican et al., 1987) animals that did not mobilize muscle protein in lactation did not change their rate of muscle protein synthesis; these animals did not lose live-weight and were in positive N balance throughout the study. Such studies would not be representative of protein metabolism in the young lactating pig. This animal typically loses fat and muscle protein tissue in lactation. However, other studies conducted in the ruminant (Bryant and Smith, 1982; Champredron et al., 1990) and rodent (Pine et al., 1994ac) indicate a decrease in the rate of muscle protein synthesis in lactating animals, provided the degree of protein mobilization is sufficiently large. Thus, a decline in the fractional rate of muscle protein synthesis contributes to the increase in protein mobilization in animals deficient in dietary protein in lactation. But the main contributor to the increase in protein mobilization observed in the rat and dairy goat in early-lactation is an increase in the rate of muscle protein degradation (Baracos et al., 1991; Tesseraud et al., 1993; Pine et al., 1994ab). This muscle process will be described in detail in this review (see Section 1.2.2).

1.2.2 Protein degradation in skeletal muscle

The degradation of myofibrillar and non-myofibrillar proteins is regulated independently and by separate pathways (Lowell et al., 1986ab). Myofibrillar proteins in skeletal muscle are sensitive to degradation in times of nutritional stress, and supply the proteins and amino acids that make up for any dietary protein deficit (see earlier in this Section 1.2). In catabolic states such as muscle denervation (Furuno et al., 1990) and starvation (Lowell et al., 1986ab; Li and Wassner, 1984) the degradation rate of myofibrillar proteins increases two to three-fold above those of animals in the fed or non-denervated state. However, the degradation rate of non-myofibrillar proteins (e.g. sarcoplasmic proteins) changes very little between the fed and fasted state.

Lysosomal and cytoplasmic proteolytic pathways exist in skeletal muscle (Furuno and Goldberg, 1986; Fagan et al., 1987; Kettlehut et al., 1988). The lysosomal proteinases include the nine identified cathepsins and other acid hydrolases and are the major site of breakdown of membrane proteins, glycoproteins such as hormone receptors, and endocytosed proteins, such as plasma proteins, hormones, and lipopolysaccharides. The lysosomal proteinases also account for the bulk of protein degradation in the liver under poor nutritional conditions (Beynon and Bond, 1986). However, they are not involved in the breakdown of myofibrillar proteins in conditions such as starvation (Lowell et al., 1986b) and muscle atrophy due to denervation (Furuno et al., 1990).

In eukaryotic cells, most proteins in the cytosol and nucleus are degraded via the ubiquitin-proteasome pathway. Ubiquitin-mediated protein degradation plays an important role in controlling basic biological processes such as cell-cycle progression, signal transduction, transcriptional regulation, receptor and transporter down-regulation and endocytosis, and degradation of abnormal proteins. The ubiquitin system has also been implicated in the immune response, development, and programmed cell death (apoptosis). Abnormalities in ubiquitin-mediated processes have also been shown to cause pathological conditions such as malignant transformation (Hershko and Ciechanover, 1998; Lecker et al., 1999a; Voges et al., 1999). These processes will not be discussed in this review of the literature. Rather, the *ATP-ubiquitin-dependent proteasome proteolytic pathway* will be discussed. The majority (65 to 80%) of muscle proteins, especially myofibrillar proteins, are degraded in this pathway (Fagan et al., 1987) in conditions of nutritional deficit.

Protein degraded in the ATP-dependent proteasome proteolytic pathway is first marked for degradation by covalent ligation to at least five ubiquitin molecules, a highly conserved protein consisting of 76 amino acid residues that is present in all eukaryotic cells (Hershko and Ciechanover, 1992, 1998). The pathway is highly selective and efficient; there are a number of ubiquitin carrier proteins (E2) and ubiquitin ligase enzymes (E3) that are specific for different protein types. Proteins are degraded in the 26S proteasome complex, a multienzyme complex that requires ATP to function (Wing and Goldberg, 1993). The importance of this process is underlined by the reduction in muscle proteolysis (sepsis-induced) in vivo by treatment with a proteasome inhibitor (Fischer et al., 2000). The ubiquitination of proteins and their subsequent degradation in the proteasome by complex mechanisms are described in Section 1.2.3.

The specificity of ubiquitin-mediated protein degradation process is probably determined to a large part by the E2 and E3 proteins, and/or by the complex of the E2 and E3 proteins. The E2 proteins are part of a protein superfamily (molecular weights ranging from 14 to 35 kDa) identified by their ability to bind to an ubiquitin affinity matrix in the presence of E1 and ATP (Pickart and Rose, 1985; Haas and Bright, 1988). Each E2 protein has a relatively specific function, and they assist in the degradation of different substrates. A number of E2 species have been found in skeletal muscle, including the 14-, 17-, and 20-kDa species, but only the 14-kDa E2 was regulated in conditions of muscle wasting (Attaix et al., 1998). The E3 proteins have less structural similarity to each other than the E2 proteins. Four types of E3s have been identified (N-end rule E3, Hect-domain E3, Cyclosome/APC, and phosphoprotein-ubiquitin ligase complex), and several others have been partially characterized (Hershko and Ciechanover, 1998; Lecker et al., 1999a)

Isolation of cDNA probes for ubiquitin, subunits of the proteasome complex (C-2, 3, 5, 8, and 9), and the 14 kDa-ubiquitin-conjugating enzyme (14 kDa-E2) has allowed changes in gene expression of this proteolytic pathway to be measured in animals in different physiological states. Increases in mRNA expression of components of the ATP-dependent proteasome proteolytic pathway were observed in muscle tissue (Medina et al., 1995) and the gastrointestinal tract (Samuels et al., 1996) of fasted rats, but not in heart, liver, spleen, fat or brain tissue (Medina et al., 1995). Up-regulation of genes in this pathway was also observed in skeletal muscle and skin of rats undergoing cancer cachexia but not in other tissues (Baracos et al., 1995).

Expression of mRNA for the 14 kDa-ubiquitin-conjugating enzyme increases upon fasting and returns to lower levels upon re-feeding, in parallel with changes in muscle ubiquitinated protein levels (Wing and Banville, 1994). In starvation and atrophy denervation the increase in protein (myofibrillar) substrates marked for degradation (ubiquitin-protein conjugates) are associated with increases in mRNA expression of ubiquitin (Wing et al., 1995). Insulin administration decreased ubiquitin gene expression in the predominant muscle fibre type degraded in catabolic conditions (mixed-fibre type), but not in slow-twitch muscle, skin, liver or jejunum (Larbaud et al., 1996). Thus, mRNA expression of ubiquitin and various proteasome subunits reflect the overall rates of muscle protein degradation in several catabolic states, and suggest that proteasome production is enhanced in skeletal muscle in animals in catabolic states. These include:

- **food deprivation and re-feeding** (Wing and Goldberg, 1993; Wing and Banville, 1994; Medina et al., 1995; Wing and Bedard, 1996)
- **muscle denervation** (Medina et al., 1995)
- **cancer cachexia** (Temparis et al., 1994; Baracos et al., 1995)
- **metabolic acidosis** (Mitch et al., 1994)
- **burn injury** (Fang et al., 1998)
- **sepsis** (Voisin et al., 1996; Tiao et al., 1997; Fang et al. 2000; Fischer et al. 2000)
- **diabetes** (Bailey et al., 1999).
- **glucocorticoid administration** (Wing and Goldberg, 1993)

No components of this proteolytic system have been measured in the pig or in lactating animals. Gene expression of this pathway has been measured in other domestic species, including the skeletal muscle of goats undergoing euglycemic hyperinsulinamia and hyperaminoacidemia (Larbaud et al., 1996), splanchnic organs of sheep undergoing underfeeding and refeeding (Nozière et al., 1999), and the muscle of growing broiler and layer chickens (Harper et al., 1999).

A number of circulating factors trigger an increase in muscle protein degradation. In denervated and immobilized muscles the signal originates within the inactive cell, whereas in various conditions of protein catabolism including fasting, acidosis, sepsis, cancer cachexia, and potentially lactation, skeletal muscle proteolysis is sensitive to hormonal regulation (Kettlehut et al., 1988; Evers, 1989; Grizard et al., 1999, Lecker et al., 1999a). In both fasting and acidosis, glucocorticoids at physiological concentrations appear to play a 'permissive role' in the catabolic response. They are required to elicit increases in the ubiquitin-proteasome ATP-dependent proteolytic system in vitro and in vivo (Wing and Goldberg, 1993; Mitch et al., 1999). The rise in proteolysis, due to starvation in vivo is blocked, and the increase in ubiquitin-protein conjugate levels is reduced by adrenalectomy (Wing et al., 1995). Similar results of adrenalectomy were observed in diabetic rats (Mitch et al., 1999). Besides promoting proteolysis in muscle, glucocorticoids and the effects of fasting also inhibit protein synthesis by decreasing translation of mRNAs encoding muscle proteins and suppressing amino acid entry into muscle (McGrath and Goldspink, 1982), and reduced translational efficiency (reviewed by Grizard et al., 1999). At the same time, glucocorticoids induce gluconeogenic enzymes that convert amino acids into glucose in the liver. Thus in fasted animals, glucocorticoids co-ordinate the mobilization of amino acids from muscle tissue, and glucose production from precursors in the liver. Protein synthesis is probably differentially regulated in muscle and liver in response to fasting and refeeding (Yoshizawa et al., 1997), and a combination of dietary protein and increased insulin concentrations may be required to stimulate translation initiation in skeletal muscle and liver tissue (Yoshizawa et al., 1998).

Ubiquitin conjugation appears to be hormonally regulated. Insulin reduced the expression of 14-kDa ubiquitin-conjugating enzyme in L6 myotube culture in vitro, but had no effect on mRNA's encoding polyubiquitin and several proteasome (C2 and C8) subunits (Wing and Banville, 1994; Wing and Bedard, 1996). But insulin administered of insulin in vivo to goats reduced ubiquitin expression in mixed fibre muscles (Larbaud et al., 1996), suggesting that insulin's anti-proteolytic effects are mediated in part by reducing muscle proteolysis. Addition of IGF-1 did not affect the rate of transcription of the 1.2 kb 14 kDa ubiquitin-conjugating enzyme transcript (active transcript) in myotube culture, but did increase the rate of degradation of this transcript (Wing and Bedard, 1996), indicating that mRNA stability of components from the proteolytic pathway are altered by IGF-1 addition.

1.2.3 Mechanism of action of the ATP-ubiquitin-dependent proteasome pathway

Proteins are ubiquitinated and subsequently degraded in the proteasome by complex mechanisms. The mechanism and substrates of this pathway and the way it influences skeletal muscle protein degradation have been comprehensively reviewed (Rechsteiner, 1991; Hershko and Ciechanover, 1992, 1998; Attaix et al., 1994, 1998; Rock et al., 1994; Mitch and Goldberg, 1996; Lecker et al., 1999a). A brief description of the process is described below. Ubiquitin is first activated by conversion of the terminal carboxyl group to a thiol ester by an ATP-requiring enzyme (E1). The activated ubiquitin is then transferred to a family of ubiquitin carrier (E2) proteins. The carboxyl group of the ubiquitin is coupled by E3 to the ε-amino group of lysines in the protein substrate. The E3 protein possesses specific binding sites for the substrate, E2 protein, and at least one (sometimes two) ubiquitin molecule(s), thus E3's active sites are able to transfer ubiquitin directly to either a lysine on the substrate or to the preceeding ubiquitin moiety to form a chain. The ubiquitin-conjugating reaction is repeated by linking activated ubiquitin to the Lys-48 residue of the substrate-bound ubiquitin. In this way a chain of five of more ubiquitin molecules is formed and linked to each other and then to the protein substrate.

The polyubiquitinated proteins are rapidly degraded in the 1,500 kDa proteolytic complex (26S proteasome), a detailed description of the structure and function of which is provided by Coux et al. (1996), DeMartino (1998), DeMartino and Slaughter (1999), and Voges et al. (1999). The 26S proteasome is composed of a 600 kDa central barrel-shaped structure called the 20S proteasome core, and has a 19S complex located at either end. The 19S complex contains 20 subunits that 1) specifically bind ubiquitin-conjugated proteins, and 2) catalyze unfolding of the proteins to facilitate their entry into the central chamber of the 20S proteasome core. These processes require energy that is provided by the six ATPase subunits present in the 19S complex. Proteolysis occurs in the 20S proteasome core that is composed in eukaryotic cells of two copies of seven distinct non-catalytic α- and seven distinct catalytic β-type subunits, arranged in four stacked rings of proteins around a central cavity. Two juxtaposed rings of β-type subunits are flanked on top and bottom by a ring of α-type subunits that form the barrel-shaped complex. Individual 19S complexes bind to each of the outer α-rings of the 20S proteasome in the presence of ATP. Unfolded protein substrates enter its core through the outer ring and are degraded by peptide-bond cleavage, catalyzed by the hydroxyl group of threonine at the N-terminal of the two inner rings. Mammalian proteasomes contain several active sites, located inside the cylinder, that differ in their substrate specificity but function together in proteolysis: one preferentially cleaves peptides after large hydrophobic, another after basic, and one after acidic amino acid residues. Once the proteins are cut into peptides of 6 to 12 amino acids they are released and hydrolyzed to amino acids by peptidases in the cytoplasm, and the ubiquitin is released and reused. The peptides (antigens) may also be transported to the endoplasmic reticulum to become involved in the production of antigenic peptides after presentation on major histocompatibility complex (MHC) class I molecules (Rock et al., 1994). The latter function of the proteasome will not be discussed further in this review. The 20S proteasome is also known as the multicatalytic proteinase, macropain, and prosome (Seemüller et al., 1998). The 19S complex or cap is also known as PA700 (Ma et al., 1994), μ-particle (Peters et al., 1994), ball (Hoffman et al., 1992), and the ATPase complex (Dubiel et al., 1995).

The ubiquitin-proteasome pathway does not degrade intact myofibrils, or complexes of these proteins (actomyosin), suggesting that actin and myosin need to be released from their myofibrils before they can be ubiquitinated and degraded by the 26S proteasome (Solomon and Goldberg, 1996). The mechanism by which myofibrillar protein is first ubiquitinated is as yet unknown. However, a calcium-dependent mechanism involving increased calpain activity has been implicated in the disruption of anchorage of actin and myosin from the Z-band (Williams et al., 1999). This may be the first step in the subsequent release of the myofilaments from the sarcomere leading to the ubiquitination and degradation of the protein.

In skeletal muscle a large proportion of non-myofibrillar protein degradation is catalyzed by the N-end rule pathway (Solomon et al., 1998a). This pathway is also important in increasing ubiquitin conjugation of skeletal muscle proteins in catabolic states (Solomon et al., 1998b; Lecker et al., 1999b). The N-end rule pathway pertains to the rapidity by which proteins are ubiquitinated and degraded. Proteins with basic (Arg, Lys, His), large hydrophobic (Phe, Leu, Trp, Tyr) or small uncharged (Ala, Ser, Thr) N-terminal residues are rapidly ubiquitinated and degraded, whereas the same proteins bearing other N-terminal residues, such as methionine, are stable (Bachmair et al., 1986; Gonda et al., 1989). These researchers speculated that exo- or endoproteolytic cleavage of long-lived proteins (actin and myosin) to expose the destabilizing amino-terminal residue is a rate-limiting step in the degradation of such proteins.

1.3 Measurement of protein synthesis and degradation

The rate of whole-body and muscle protein synthesis and degradation may be measured in vivo by a number of methods that are based on the concept of amino acid flux, and rely on the assumption that the free amino acid pool is kinetically and metabolically homogeneous. The first method is based on a single tracer dose of a stable or radioactively labeled amino acid such as phenylalanine, tyrosine, or methionine. This method is not manageable within an experimental setting because of the frequent sampling and large sample size required. Second, a constant infusion of a labeled amino acid such as tyrosine, leucine, phenylalanine, and methionine for up to 11 h may be used. This method provides a good measure of protein synthesis rates in tissues with a slow rate of turnover such as skeletal muscle, but not for tissues with high protein turnover rates and which export a large proportion of their synthesized protein. This method has been used in large domestic species such as cattle (Lobley et al., 1980; 2000), and goats (Champredon et al., 1990; Baracos et al., 1991). A third method involves administration of a tracer dose of labeled amino acid, such as phenylalanine and valine, with a large dose of carrier unlabelled amino acid to "flood" the free amino acid pool, and thereby minimize the differences between the extracellular and intracellular free amino acid isotope abundance (Reeds, 1992). This method has been used to measure muscle protein turnover in lambs (Attaix et al., 1986) and pigs (Mulvaney et al., 1985; Seve et al., 1993). These methods are expensive to conduct, have numerous sources of error, and cannot be repeated multiple times on the same animal. In vitro measures of muscle protein turnover can also be made in small animals, such as rats, but are not possible in larger animals because a small muscle cannot be excised intact (Tischler, 1992).

Other methodologies used in fields of research that face similar technical problems to those seen in the lactating sow measure indices of muscle protein synthesis and degradation which involve minimally invasive techniques. The study of protein breakdown in critically ill and (or) post-operative patients is such a field of research. A parallel can be drawn between the metabolic situation experienced by the lactating sow and by critically ill patients that exhibit catabolism and negative nitrogen balance due to excess muscle protein catabolism. In both cases there is an imbalance between protein supply from endogenous muscle protein catabolism and the overall demand for amino acids. Furthermore, in both cases studies of protein synthesis and breakdown rate preclude the use of determinations of protein depletion that are invasive, use of radioisotopes or are conducted in facilities remote from the subject (farrowing barn or hospital). Research in this area has revealed several indices of muscle protein breakdown that are considered diagnostic of catabolic states in which protein mobilization occurs. These have been documented in a diversity of physiological states where there is disease and/or malnourishment in the human patient and include changes in muscle RNA:DNA and protein:DNA ratio, free amino acid concentration and mRNA expression of the ATP-dependent ubiquitin proteasome proteolytic pathway and are described below (see Sections 1.3.1 to 1.3.3). Other methods of estimating muscle protein synthesis and degradation are found in the field of animal science and these include urinary 3-methylhistidine excretion (3MH; see Section 1.3.4) and myofibrillar protein degradation using compartmental-kinetic analysis of de novo 3MH synthesis (see Section 1.3.5).

1.3.1. Muscle RNA:DNA and protein:DNA ratio

The growth of tissues and organs in terms of increases in total DNA (hyperplasia) and protein:DNA ratio (hypertrophy) are well documented. This relationship is more complex in skeletal muscle because each muscle fibre is comprised of one or a small number of multinucleate cells. Growth in muscle tissue involves the addition of nuclei produced by mitoses in a population of satellite cells that are located between the plasma membrane of the muscle cell and the basement membrane surrounding and supporting the individual fibres. To account for the multinucleate nature of muscle, the muscle cell has been defined in operational terms as the volume of cytoplasm controlled by a single nucleus i.e. a DNA unit (Cheek et al., 1971). The amount of DNA in skeletal muscle increases with age, but after about 5 mo of age porcine muscle DNA concentrations remain fairly constant (Powell and Aberle, 1975). This allows muscle DNA concentrations to be used as an index of skeletal muscle 'cell number' in the adult animal such as the lactating sow. The muscle protein:DNA ratio therefore estimates the amount of protein per muscle 'cell unit'. Changes in this ratio in the adult animal are indicative of a change in muscle cell 'size', and ultimately in the body's protein reserves. This is demonstrated by the 50% reduction in the mixed-fibre muscle (plantaris) protein:DNA ratio and wet weight in rats that were restrict fed (Sika and Layman, 1995).

Ribosomal RNA is the cell's protein synthetic machinery. The majority (> 80%) of total tissue RNA is ribosomal (Darnell et al., 1986), and about 80% of ribosomes translate message at any given time (in the liver; Scornik, 1974). Then by relating total tissue RNA concentrations with their DNA concentration (RNA:DNA ratio) the protein synthetic capacity of the tissue 'per muscle cell unit', can be estimated. The RNA:DNA ratio is therefore used as an index of the rate of muscle protein synthesis. It is highly correlated with changes in the rate of muscle protein synthesis in starvation and after feeding a protein-free diet for several days in the rat (Millward et al., 1974; Goodman and Ruderman, 1980). Also daily changes in this ratio are observed due to meal-feeding and parallel the changes in plasma insulin (Millward et al., 1974). Ribosomal capacity (RNA:protein ratio) may also be used as an index of the rate of protein synthesis (Millward et al., 1974).

1.3.2 Muscle free amino acid concentrations

The muscle free amino acid pool constitutes the main free amino acid pool in the body (Young, 1970). It reflects the balance between the net rate of amino acid appearance from myofibrillar protein breakdown, and the net rate of amino acid disappearance due to either export out of the tissue into the blood, re-synthesis into tissue protein, or other tissue metabolism uses. In contrast, plasma free amino acid pool represents a small proportion of the whole-body free amino acid pool and is the medium by which free amino acids are transported around the body to peripheral tissues. In lactation, amino acids are predominantly exported to the mammary gland for milk biosynthesis and other mammary functions.

If the dietary protein intake is inadequate to provide for the animal's requirements there is a net efflux of amino acids out of muscle tissue. A reduction in free glutamine concentrations and increases in the branched chain amino acids (BCAA's, isoleucine, leucine and valine) and phenylalanine concentrations in muscle and plasma are diagnostic of muscle protein mobilization in the critically ill and(or) post-operative patient (Table 1.1). Similar changes in the pattern of the muscle free amino acid pool have been observed in other catabolic states. These include lactation in the dairy cow (Motyl et al., 1983; Meijer et al., 1995), glucocorticoid administration to rats, and several days of starvation or of feeding a protein-free diet in the rat (Millward et al., 1974, 1976). Changes in the free muscle amino acid pool are often larger and more numerous than those in the plasma pool (Table 1-1), suggesting that this pool is more sensitive to loss of body protein than the free plasma pool.

1.3.3. The ubiquitin-ATP-dependent proteasome proteolytic pathway

The ubiquitin-ATP-dependent proteasome proteolytic pathway is the main proteolytic pathway in muscle (Attaix et al., 1998). Increases in mRNA expression of genes from this pathway are related to increases in the rate of muscle protein degradation in a number of physiological conditions including starvation, cancer cachexia, metabolic acidosis, sepsis, burn injury and diabetes (see Section 1.2.2). However, some caution must be used if this is the sole index of the rate of muscle protein degradation used in an experiment. Under some physiological conditions changes in the level of mRNA expression for various aspects of the ATP-dependent ubiquitin proteasome proteolytic pathway do not always reflect the rate of muscle protein breakdown. Peripheral treatment of septic rats with IGF-1 prevented the increase in ubiquitin and 14-kDa ubiquitin-conjugating enzyme mRNA expression in muscle, but did not reduce the rate of the total and myofibrillar protein breakdown (Fang et al. 2000). The IGF-1 treatment probably reduced the sepsis-induced muscle cachexia by stimulating the

Table. 1-1 Change in muscle and plasma free amino acid concentrations after elective-surgery, during sepsis or critical illness in human patients

		Changes in Free Amino Acid			
		Essential amino acids		**Non-essential amino acids**	
Reference	**Treatment**	**Plasma**	**Muscle**	**Plasma**	**Muscle**
Vinnars et al. (1975)	Post-surgery (3 d)	-38% His -9% Ile +25% Leu +60% Phe	-47% Arg -42% Lys +92% Val +100% Phe +113% Leu	-88% Tyr -29% Pro -17% Ser	-38% Gln -22% Glu +30% Ala +30% Tyr +37% Ser +47% Gly
Askanazi et al. (1980)	Post-surgery (4 d)	-22% His +33% Val +49% Phe +60% Leu +65% Ile	-24% His +83% Trp +92% Val +118% Phe +128% Leu +209% Ile +261% Met	-34% Ala -29% Gly	-49% Gln
Milewski et al. (1982) (Data derived from graphs)	Septic & malnourished patients	-40% His -10% Ser	-40% Lys -30% His +50% Val +100% Leu +100% Ile +100% Phe +100% Met	-30% Thr +80% Phe	-80% Gln -40% Arg +30% Ser +30%Gly +60% Asn +100% Tyr
Hammarqvist et al. (1989)	Post-elective abdominal surgery (3 d)	+36% Phe	-39% Arg -20% His +28% Thr +57% Leu +67% Ile	-23% Glu +33% Asp	-78% Glu -61% Gln -50% Cys +89% Ser
Petersson et al. (1992)	Post-elective abdominal surgery (3 d)	-33% Leu -9% His +30% Thr +59% Phe +60% Ile +65% Met +78% Lys	+56% Phe +75% Ile	+57% Tyr	-38% Gln -37% Glu +39% Asn
Gamrin et al. (1997)	During critical illness		+20% Val +33% Leu +44% Phe		

rate of muscle protein synthesis. The deviation from the relationship between muscle protein degradation and up-regulation of gene expression of this pathway was observed after hormonal intervention. However, under conditions of nutritional deprivation increases in mRNA expression of components of the ATP-ubiquitin-dependent proteasome pathway in muscle is highly related to increases in the rate of muscle protein degradation. The assumption is made that under these conditions the increased mRNA expression observed reflects an increased transcription rate and no change in mRNA stability.

1.3.4 Urinary excretion of 3-methylhistidine

Muscle protein degradation can be measured by urinary excretion of 3-methylhistidine in many animal species, if the intake of dietary 3-methylhistidine is accounted for (Young and Munro, 1978). This unusual amino acid is a minor constituent of actin in all muscle types and in the myosin heavy chain in fast-twitch glycolytic fibres, and is therefore present in a set ratio in skeletal muscle. It is formed by post-translational methylation of histidine residues and is released when skeletal muscle is broken down because 3-methylhistidine does not have a specific tRNA and is not reincorporated into protein (Young et al., 1972), and it is also not catabolized. Therefore, urinary 3-methylhistidine excretion is assumed to represent the rate of myofibrillar protein breakdown, and to be representative of the breakdown of other proteins in muscle which do not contain it. However, in certain species including the pig and sheep, red deer, horse and sperm whale, 3-methylhistidine is not quantitatively excreted in urine. In these species, once free 3-methylhistidine is released from muscle protein it predominantly forms a dipeptide with β-alanine called balenine, and is recycled or stored in the body rather than excreted in the urine (Harris and Milne, 1981). Therefore urinary 3MH excretion cannot be used to estimate skeletal muscle protein breakdown in these species, which included the pig.

The majority of 3-methylhistidine is located in skeletal muscle, and the remainder is located in the smooth muscle of the gastrointestinal tract and skin (Millward and Bates, 1983). The rate of turnover of myofibrillar protein in the gastrointestinal tract is about 18 times greater than that in the skin and muscle (Wassner and Li, 1982; Millward and Bates, 1983). Therefore, skeletal muscle only accounts for 38 to 60% of the urinary 3-methylhistidine excretion in the fed state. The remainder of the excretion is contributed by the gastrointestinal tract (22 to 40%) and smooth muscle in the lungs and vascular system (up to 26%) (Wassner and Li, 1982; Millward and

Bates, 1983). Therefore, urinary 3-methylhistidine excretion is not the best method for estimating the extent of muscle protein degradation, because it is not possible to distinguish between muscle-derived 3-methylhistidine and that from other sources. Furthermore, 70 to 100% of 3-methylhistidine occurs in actin, depending on the relative amounts of the different forms of myosin heavy chain present in the muscle. The synthesis rates of protein-bound 3-methylhistidine primarily reflect the synthetic rates of actin, which has a very long half-life (~ 15 d) and this adds error to the measurement. However, degradation of actin is highly sensitive to changes in metabolic status, so although the rate of actin breakdown is not representative of breakdown of other muscle proteins, it does increase significantly in catabolic states.

1.3.5 Myofibrillar protein degradation

Recently an alternative methodology has been developed to measure the rate of muscle protein degradation in species, such as the pig, in which urinary 3-methylhistidine excretion cannot be used (see Section 1.3.4). This technique measures myofibrillar protein degradation using compartmental-kinetic analysis of de novo 3-methylhistidine synthesis (Rathmacher et al., 1992ab). It has been used to directly measure myofibrillar protein breakdown in cattle (Rathmacher et al., 1992b), sheep (Rathmacher et al., 1993) growing pigs (van den Hemel-Grooten et al., 1995; Rathmacher et al, 1996), as well as lactating pigs (Trottier et al., 1995; Jones and Stahly 1999a, Yang et al., 2000b). However, this technique involves urinary catheterization and is fairly expensive.

1.4 Body protein mobilization in the lactating pig

The amount of protein tissue mobilized from a sow in lactation has been measured in comparative slaughter experiments either as whole-body protein, including all the organs, or as muscle protein dissected from the primal cuts (shoulder, ham, loin, and belly) of the carcass (Table 1-2). From these measures of body protein mass, equations have been formulated based on non-invasive measures that may be made in real time over the course of lactation (live-weight and backfat depth) that predict the sow's protein mass (Table 1-3). These equations account for a large proportion of the variation (67 to 91%) in the predicted whole-body protein and muscle mass in reproductive female pigs of various body sizes and stages of their cycle. Similar equations have been formulated to predict the sow's whole-body fat mass and fat mass dissected from the primal cuts of the carcass; these equations also account for a large proportion of the variation (80 to 95%) in the predicted fat mass (Table 1-3).

Deuterium oxide dilution is another method used to estimate the protein or muscle mass of a lactating and/or gestating pig. Equations formulated based on this variable and the sow's whole-body protein and fat mass are fairly accurate at predicting whole-body fat ($R^2 > 0.70$) but not protein mass ($R^2 > 0.23$) in reproducing female swine (Shields et al. 1984). Measurement of the intracellular pool of 3-methylhistidine, by the 3-methylhistidine production by compartmental analysis (see Section 1.3.5), also

Table 1-2 Summary of experiments used to predict body protein/muscle and lipid mass in gestating/lactating sows

Reference	Tissues	Animals	Time of Slaughter
Wałach et al. (1986b)	Whole-body protein & fat mass (- blood & bristles)	Landrace (Gilt)	At breeding (n = 28)* d 112 gestation (n = 37)
Whittemore &Yang (1989)	Whole-body protein & fat mass [a]	Large White x Landrace [b] (Gilts, 1st & 4th parity sows)	Gilt breeding (n = 6)* 1st parity wean (n = 12)* 14 d after 4th parity wean (n = 24)*
Mullan & Williams (1990)	Whole-body protein and fat mass [a]	Landrace x Large White (1st parity sows)	Sows at various stages of lactation & undergoing differential protein/fat loss (n = 73)*
Everts & Dekker (1995)	Whole-body protein & fat mass (- hair)	Large White x Dutch Landrace (Gilts & 3rd parity sows)	At breeding (n = 11)* d 108 gestation (n = 14)* 1 wk post-wean 3rd wean (n = 23)*
Dourmad et al. (1996)	Muscle mass (incl. intermusclar fat) & dissectable fat [c]	Large White (Multiparous sows)	~1 wk post-wean (n = 24)* d 112 gestation (n = 21)
Dourmad et al. (1998)	Muscle mass (incl. intermusclar fat) & dissectable fat [c]	Large White (1st parity sows)	At farrowing (n = 30)* At weaning (n = 43)

[a] Whole body protein/fat mass included carcass, head, feet, tail, viscera, blood, skin and hair.

[b] Source of pigs for this experiment was Cotswold Pig Development Company Ltd.

[c] Muscle mass measured as the muscle tissue dissected from cut-out from the carcass primal cuts

* Denotes animals used to formulate prediction equation.

estimates the body's muscle mass, but this method is also fairly inaccurate ($R^2 = 0.35$; Rathmacher et al., 1996). Additional drawbacks to these techniques, other than their inaccuracies, are the requirement for venous catheterization of the sow for infusion and blood collection. Another approach uses relative changes in urinary creatinine excretion during the course of lactation. Creatinine is formed as the end-product of irreversible muscle creatine metabolism, and is eliminated from the body in urine (Bingham and Cummings, 1985). Urinary creatinine is often used as an indicator of the body's muscle mass because the amount excreted is thought to depend on the body's muscle creatine content (Blackburn et al., 1977). Thus, changes in maternal

Table 1-3 Equations to predict whole-body protein and fat mass or dissectable muscle and fat mass

	Equation	R^2
From Walach et al. (1986b): Whole-body (kg)		
Protein	1.58 + 0.15 LW – 0.11 BF	0.72
Fat	5.85 + 0.07 LW + 1.06 BF	0.66
From Whittemore &Yang (1989): Whole-body (kg)		
Protein	-2.31 (±1.57) + 0.186 (± 0.007) LW – 0.216 (± 0.074) BF (P2)	> 0.90
Fat	-20.4 (± 4.46) + 0.205 (± 0.019) LW + 1.48 (± 0.210) BF (P2)	> 0.80
From Mullan & Williams (1990): Whole-body (kg)		
Protein	4.46 (± 0.982) + 0.11 (± 0.012) LW – 0.13 (± 0.037) BF (P2)	0.67
Fat	-31.1 (± 2.625) + 0.381 (± 0.031) LW + 1.042 (± 0.098) BF (P2)	0.95
From Everts & Dekker (1995): Whole-body (kg)		
Protein	1.67 (± 1.32) + 0.175 (± 0.008) LW – 0.377 (± 0.053) BF (P2)	0.91
Fat	-10.40 (± 2.80) + 0.110 (± 0.017) LW + 1.997 (± 0.112) BF (P2)	0.92
From Dourmad et al. (1996): Dissectable tissue mass (kg)		
Muscle	-9.2 + 0.61 (± 0.052) LW – 0.86 (± 0.29) BF (P2)	0.89
Fat	-21.3 + 1.51 (± 0.297) BF (P2) + 0.29 (± 0.084) AD	0.92
From Dourmad et al. (1998): Dissectable tissue mass (kg)		
Muscle	-4.5 + 0.534 (± 0.056) LW – 0.44 (± 0.29) BF (P2)	0.82
Fat	-13.8 + 0.10 (± 0.03) LW + 1.08 (± 0.10) BF (P2)	0.83

Where LW = sow live-weight (kg); BF = mean backfat at the last rib 3 and 8 cm from the dorsal midline (mm); BF (P2) = backfat at the P2 site (mm); and AD = average adipocyte diameter (mm). The dissectable muscle mass from cut-out of the carcass primal cuts (shoulder, ham, loin and belly) including intermuscular fat.

muscle protein mass, would be reflected in the amount of creatinine excreted in urine (Tietz, 1986). This technique could be used in conjunction with nitrogen balance studies as urinary catheterization is required.

Nitrogen balance has also been used to measure body protein loss in the lactating pig (Noblet and Etienne, 1987; King et al., 1993a; Everts and Dekker, 1994ab; Dourmad et al., 1998), human (Motil et al., 1990), and dairy cow (Botts et al., 1979). This technique allows sequential measurements to be taken but it overestimates N intake and underestimates N loss with the resultant effect that is overestimates the actual N balance (Just et al., 1982; Manatt and Garcia, 1992; Dourmad et al., 1998). Account is not made of other routes of N loss such as endogenous losses, integumental losses (hair, skin, and nail loss and growth and sweat loss) and other miscellaneous losses (Pellett, 1990; Manatt and Garcia, 1992). Other draw-backs to this technique include urinary catheterization, which introduces a potential route of infection, and the fact that in the lactating pig loss of N in milk can only be estimated. Due to these inherent inaccuracies, the comparative slaughter technique is a better predictor of changes in the protein mass of a sow over longer time periods (over three wk), and when large changes in body protein mass are observed. Nitrogen balance is better able to measure changes over a short time periods when small changes in the body protein mass may not be picked up by the comparative slaughter technique (Everts and Dekker, 1994a; Dourmad et al., 1998). Even so, the majority of these techniques fail to distinguish between the size of the sow's muscle protein mass and protein in the rest of the body. Even the experiments that measured the dissected mass of muscle tissue from primal cuts included intermuscular fat as a component of muscle mass. This increases the estimated value of muscle mass by 10% (Martin et al., 1981). They also included a primal cut (belly) that contains a large proportion (14%) of intermuscular fat, and in which the amount of intermuscular fat increases with animal fatness (Martin et al., 1981). The equations that estimate muscle mass of Dourmad et al. (1996, 1998) are therefore confounded by fat mass.

Because of the associations between loss of an animal's muscle protein reserves and lactational and reproductive function it would be advantageous to measure the amount of muscle tissue in a carcass, possibly by dissection of the lean cuts. Equations could be formulated to predict the sow's muscle mass, and changes in her muscle mass during lactation, from non-invasive measures that can be measured consecutively such as live-weight, backfat depth and muscle parameters such as loin muscle depth and area. An alternative approach to measuring changes in the sow's muscle mass during lactation requires the technique of percutaneous muscle biopsy that can be repeated on the same individual multiple times. This removes between animal variance and allows establishment of temporal changes in muscle composition indices of muscle protein synthesis and degradation. (see Sections 1.3.1 to 1.3.3).

1.5 Gestation

In order to meet the nutritional requirements of the sow in gestation, an understanding of maternal growth, and the needs for adequate growth and development of the fetus and mammary gland is required. Several researchers have studied maternal growth in the gestating pig, and they suggest that pregnancy anabolism occurs in the pregnant pig (Hovell et al., 1977; Close et al., 1985; Wałach-Janiak et al., 1986b). Increases in the gilt's whole-body (35.8 to 91.2 g/d) and maternal (17.7 to 70.8 g/d) protein deposition occur upon feeding increasing digestible protein (181 to 420 g/d) and energy (18.9 to 44.1 MJ ME/d) intakes during gestation (Wałach-Janiak et al., 1986a). Similarly, increasing the gestational energy intake from 19.5 to 32.1 MJ ME/d, and keeping crude protein intake constant (310 g/d), increased maternal protein deposition in gestation from 24 to 49 g/d (Hovell et al., 1977). However, because of the high demands of the developing fetus in the last trimester of gestation (see Section 1.5.1) maternal skeletal muscle protein mobilization can occur if the nutrient supply of the dam is not increased. This has been observed in the gestating rat fed a low protein diet (7.5 vs 25% casein diet) (Zartarian et al., 1980). It has also been indicated in the gestating pig by progressive increases in blood alanine concentrations between d 85 to 115 of gestation (Simoes Nunes et al. 1987).

1.5.1 Fetal growth and development

The most rapid fetal gains (50 to 60% of the total gain) occur in the last trimester of gestation, after d 90 in the pig. This is illustrated by a four-fold increase in the rate of fetal growth (66 vs 290 g/d) between d 50 and 110 of gestation (Knight et al., 1977; Noblet et al., 1985). In late pregnancy the placenta is also highly metabolically active; the non-fetal component of the gravid uterus consumes 35 to 50% of the oxygen, at least 65% of the glucose, and more than 50% of amino acids, especially BCAA's, glutamine and citrulline in sheep and cattle (Bell, 1995).

Growth and development of the fetus is very well protected in utero. However if nutrient restriction is severe in gestation fetal growth will be impaired. Because glucose is transported across the placenta via facilitated diffusion it is responsive to changes in maternal glycaemia. So in late gestation when glucose uptake may be reduced, a deficit in the availability of glucose for oxidation is made up by increased amino acid catabolism. However it is possible that this occurs at the expense of protein synthesis and deposition in fetal tissues resulting in reduced growth. Maternal malnutrition in rats fed 50% of ad libitum rather than ad libitum in the last trimester of gestation (week) resulted in fetal growth retardation, regardless of the maternal body weight gain and nutrient intakes in the previous two trimesters (Anderson et al., 1980). When additional nutrients become available in the last trimester, the fetus appears to preferentially receive those nutrients (Anderson et al., 1980). Thus the female rat is protected against severe depletion of maternal nutrient stores if dietary restriction is imposed.

Amino acid uptake by the placenta is largely independent of changes in maternal blood concentrations because amino acids are moved across the placenta by active transport. Even feeding a protein intake as low as 164 g/d to gestating gilts did not affect piglet birth weight (Mahan and Mangan, 1975). Feeding rats a low compared to a high protein diet (7.5 vs 25% casein diet) in early gestation (d 4 to 12) and in the last trimester (d 14 to 21) did not affect fetal weight or number (Zartarian et al., 1980). However fetal growth and development retardation can occur if animals are fed very low feed or protein intakes in gestation, resulting in smaller less viable offspring. Feeding an essentially protein-free (9 g CP/d) diet to gilts for all, or the majority (d 24 to 115) of gestation reduced piglet birth weight but did not affect the number of piglets born and born alive or the gestation length (Pond et al., 1968, 1969). Birth weight and postpartum survival were also reduced in the progeny of gilts fed 91 compared to 255 g CP/d in gestation (Shields et al., 1985).

Subsequent growth of the progeny can also be affected by the gestational nutrition of the dam. About a 10% increase in the number of secondary muscle fibres was observed in the progeny of gilt's fed a higher intake (5.0 vs 2.5 kg/d) in early gestation (d 25 to 50) (Dwyer et al., 1994). Conversely, under-nutrition in gestation inhibits fetal muscle development and fibre number in the guinea-pig (Dwyer and Stickland, 1994; Dwyer et al., 1995) and sheep (McCoard et al., 1997). Mild postnatal under-nutrition also appears to influence muscle development at the molecular and functional level in the weaned pig (White et al., 2000).

1.5.2 Mammary gland growth and development

Development of the mammary gland has been reviewed in ruminants and rodents (Tucker, 1987; Akers, 1990; Baldwin and Miller, 1991; Knight and Wilde, 1993) and the pig (Hartmann et al., 1995). Unlike other major organs, the mammary gland undergoes very limited structural development in utero, with the most dramatic changes occurring in the reproductive cycle (pregnancy, lactation, and weaning), and the majority (48 to 94%, dependent on the species) of growth occurs in gestation (Tucker, 1987; Baldwin and Miller, 1991). At birth this gland largely consists of connective tissue, and has an immature, poorly developed duct system that remains largely quiescent until the onset of puberty, and ovarian activity. The beginning of the lactation cycle involves further development of the mammary gland (mammogenesis), initiation of milk synthesis (lactogenesis 1) and secretion (lactogenesis 2), lactation (galactopoiesis), and finally regression of the mammary gland after weaning (involution) (Hartmann et al., 1995).

In response to the hormonal stimulation of puberty and the ensuing reproductive cycles, mammary stromal tissue proliferates and the ducts elongate into the stromal portion of the mammary gland. The rapid allometric growth of mammary tissue in gestation is caused by an increased and synchronous secretion of estrogen and progesterone (Baldwin and Miller, 1991; Tucker and Serjsen, 1993), and the coincidental secretion of prolactin and possibly growth hormone. In general, estrogen stimulates mammary duct growth and progesterone regulates lobulo-alveolar (milk secretory apparatus) development. Prolactin and growth hormone must be present to enable these steroids to stimulate mammary growth (Tucker and Serjsen, 1993). Prolactin is essential for normal mammary development in the pig; treatment of gilts with bromocriptine from d 70 of gestation reduced mammary weight, and total DNA and RNA content and the number of higher affinity prolactin receptors on mammary parenchymal tissue on d 110 of gestation (Farmer et al., 2000).

In the pig, as in other species, progressive mammary duct growth occurs early in gestation but very little mammary cell proliferation occurs before d 50 (Hacker and Hill, 1972). Between d 75 and 90 of gestation the most rapid mammary tissue growth takes place, and lobulo-alveolar development occurs to complete mammogenesis (Kensinger et al., 1982). From mid- (d 50 to 75) to late-gestation (d 90 to 105) mammary parenchymal (secretory epithelial cells) tissue more than doubles and there is a concomitant reduction (60%) in parenchymal lipid (Weldon et al., 1991). Mammary protein synthetic capacity (RNA:DNA ratio) also increases with mammary development (Hacker and Hill, 1972). This is associated with large increases in total mammary DNA (4 to 5-fold), parenchymal DNA (7-fold), and dry, fat-free tissue (4 to 7-fold) (Hacker and Hill, 1972; Kensinger et al., 1982; Weldon et al., 1991).

The onset of lactatogenesis occurs between d 90 and 105 of gestation, as the mammary tissue differentiates and changes from the non-lactating to the lactating state. This leads to a dramatic change in the appearance and chemical composition of this tissue (Kensinger et al., 1982; Shield and Mahan, 1983). The alveoli become extremely distended as secretory products enter the lumen by d 112 of gestation. By d 4 of lactation the alveoli appear highly differentiated, secretory epithelium occupy most of the gland and few adipose or other connective tissue cells are present in the mammary tissue which is mainly occupied by secretory epithelium. The protein synthetic capacity of the mammary tissue almost triples between d 90 of gestation and d 4 of lactation (Kensinger et al., 1982), indicating a dramatic increase in the biosynthetic capacity of the gland, readying it for milk production. If lactogenesis is initiated early (d 102 of gestation) by administration of prolactin, a reduction in the subsequent milk yield and alteration in colostrum composition occurs (King et al., 1993c).

1.5.3. Effect of gestational nutrition on mammary gland growth and development.

Feeding high energy levels to sows in gestation has equivocal effects on mammary development and mammogenesis. Mammogenesis was reduced by feeding gilts a high compared to a control energy intake (43.9 vs. 24.1 MJ ME/d) from d 75 of gestation. The animals fed the high energy intake had a lower mammary parenchymal weight, total mammary DNA and RNA content (~30%), and mammary protein content (60%) by d 105 of gestation (Weldon et al, 1991). An Australian research group observed a similar effect of feeding a high energy level in gestation (36.7 vs. 23.0 MJ ME/d). By d 112 of gestation gilts fed the high energy level had half the mammary alveolar cells (Head et al., 1991) and a quarter the mammary DNA concentration (Head and Williams, 1991). To maximize milk production in the two animal groups, the animal's litter was replaced within 24 h of parturition with nine 14-day old pigs to intensify the suckling stimulus. Litter growth rate was 50% slower in the sows fed the high energy level (Head and Williams, 1995), probably because of the fewer potential milk secretory cells present in the mammary gland at the onset of lactation. However, in this experiment gilts fed the high energy level had lower protein intakes (145 vs 269 g/d) and were obese at the end of gestation (P2 backfat depths ~35 vs ~26mm) (Head and Williams, 1991; Head and Williams, 1995). This could have contributed towards the reduced mammary development. This Australian research group has since failed to repeat the reduction in mammary gland development (Smits et al., 1995) and litter growth rate (Smits et al., 1995; Revell et al., 1998) observed by feeding high energy intakes in gestation (32.2 vs. 23.7 MJ ME/d). Mammogenesis was also unaffected by feeding gilts very divergent levels of energy intake (25.5 or 43.9 MJ ME/d) in gestation; total mammary parenchymal DNA and RNA levels in late gestation (d 105) did not differ among treatments (Howard et al., 1994).

In contrast, no adverse effects of high lysine and protein intakes in gestation have been observed on mammogenesis in the pig. Mammary DNA concentrations on about d 105 of gestation were similar in gilts fed divergent total lysine (4 to 17 g/d) and crude protein (104 to 330 g/d) intakes throughout or for various periods of gestation (Weldon et al., 1991; Kusina et al., 1999a; Smits et al., 1995). No difference in mammary DNA concentration was observed in mid- (d 14) and late- (d 28) lactation between animals fed divergent protein intakes (133 to 265 g/d) in gestation (Smits et al., 1995). Only by feeding very low protein intakes in gestation has mammary development been inhibited. Gilts fed a very low protein level (~9 g/d) in gestation had lower litter growth rates in lactation, independent of whether the litters were from sows fed the low protein or control diet (Pond et al., 1968). Similarly, lower litter growth rates were observed in gilts fed low compared to adequate protein intakes (91 vs. 255 g/d) in gestation (Shields et al., 1985). This was likely the result of a number of factors including reduced mammogenesis in the sows and their smaller, less viable offspring (see Section 1.5.1) that would provide a less intense suckling stimulus which could potentially lower milk yield (see Section 1.6.2). Mammogenesis was also reduced in rats that were restricted-fed (50% ad libitum) rather than ad libitum fed from d 5 of gestation (Rosso et al., 1980); at the end of gestation mammary gland weight, parenchymal cell number, and mammary RNA, DNA and protein content were lower in the restricted-fed rats.

1.6 Lactation

1.6.1. Control of milk production

Milk production in the sow is controlled by both endocrine (hormonal) and autocrine (local) factors (Hartmann et al., 1995; Boyd et al., 1995). These include first, the number of secretory cells present in the mammary gland at parturition and during lactation (Hartmann et al., 1995). This is under endocrine control and can be affected by the sow's gestational nutrition (see Section 1.5.3). Second the degree of glandular stimulation and milk removal by the progeny (see Section 1.6.2). Third the availability of milk precursor to, and uptake by, the mammary gland (see Section

1.6.3). Fourth, is the coordinated partitioning of nutrients to the mammary gland from dietary sources and extra-mammary tissues such as muscle and adipose tissue.

Maintenance of milk production requires a mixture of several hormones, including glucocorticoids, prolactin and oxytocin. Some enhancement of milk production can be achieved with the addition of long-acting insulin, estrogen, triiodothyronine, and growth hormone (Baldwin and Miller, 1991). Prolactin regulates the rates of transcription of genes for milk proteins and increases the half-lives of the resulting mRNA's. Glucocorticoids enhance the effect of prolactin on the rates of transcription of the casein genes (Baldwin and Miller, 1991). Administration of bovine somatotropin (bST) to lactating dairy cows increases peak milk yield and the persistency of this yield over the whole of lactation, and is accompanied by no change in gross milk composition if the nutritional intake of the treated animals is adequate. This increase in milk production is due to an increase in the rate of protein synthesis in the mammary gland and possibly to an increase in mammary epithelial cell number (Bauman and Vernon, 1993). Daily administration of porcine somatotropin (pST) to the lactating sow either from d 100 of gestation throughout lactation (Spence et al., 1984), or from d 12 of lactation (Harkins et al., 1989) increased milk yield at the end of lactation by 15 and 22%, respectively, without altering gross milk composition. However, despite the increase in milk production a 12 to 20% reduction in lactational feed intake was observed from the start of the pST administration in the treated sows (Spence et al., 1984; Harkins et al., 1989). This resulted in substantially larger weight and backfat losses in these lactating animals, but no difference in plasma NEFA levels (Harkins et al., 1989). Plasma glucose concentrations at d 29 of lactation were elevated in the pST treated sows, which may account for the differences in feed intake observed (Harkins et al., 1989). But other factors such as leptin may also be involved.

1.6.2 Mammary gland growth in lactation

Mammary tissue growth continues until mid- to late- lactation in the pig. Hyperplasic growth (cell differentiation) accounts for a portion of the lactational mammary gland growth, when parenchymal tissue replaces fat in the gland, and hypertrophic growth (cell proliferation) accounts for the remainder of the growth (Kim et al., 1999a).

Between d 5 and 21 of lactation an increase in mammary gland wet weight (55%), DNA content (100%), and protein and dry fat free tissue weight (60%) was observed, with a concomitant decline in the fat content of the mammary gland until d 14 (Kim et al., 1999a). Similarly in ruminant and rodent species, mammary gland growth can continue into established lactation (Knight and Wilde, 1987, 1993; Sejrsen and Purup, 1994). This is mainly due to hypertrophic growth in early lactation, but some hyperplasic growth also occurs. (Knight and Wilde, 1993).

Removal of milk from the mammary gland is the most important factor involved in maintaining milk secretion in mammals. If milk removal is not complete premature mammary tissue involution is initiated which ultimately reduces milk production. Mammary gland growth in lactation and the degree of milk removal is influenced by the degree of mammary gland stimulation. This can be achieved through increasing:

1) the suckling stimulus by increasing litter size in the rat (Tucker, 1964; Tucker, 1987) and pig (Auldist et al., 1995, Kim et al., 1999b)
2) suckling frequency in rodents (Knight and Wilde, 1987) and pigs (Sauber et al., 1998)
3) milking frequency in goats (Knight and Wilde, 1987).

The increased mammary gland stimulation ultimately results in an increase in milk production in the goat (Knight, 1987), and increased litter growth rate in the pig (Auldist et. al., 1995) and rodent (Tucker, 1964). And is achieved by stimulating suckling and(or) milking-induced neuronal pathways, stimulating the release of suckling-associated galactopoietic hormones such as prolactin, and removing from the gland milk and any bioactive factors present in the milk (Knight and Wilde, 1993). Prolactin is released upon tactile stimulation of the teat, such as nuzzling and suckling by the piglet, leading to a doubling of peripheral concentrations almost immediately (van Landeghem and van de Weil, 1978). In the pig, peripheral prolactin levels increase 72 h pre-partum, peak the day before parturition (de Passillé et al., 1993), gradually decrease as lactation progresses (Farmer et al., 1998) and fall rapidly within a few hours of weaning (van Landeghem and van de Weil, 1978; Foxcroft et al., 1987). The gradual decrease in peripheral prolactin levels is likely due to an extension of the suckling interval and reduced stimulation of the mammary gland by the progeny between suckling bouts as lactation progresses.

There is therefore scope to increase the sow's milk yield nutritionally by:

1) optimizing gestational nutrient and energy intake to support normal or improved fetal and mammary gland growth and development
2) optimizing the lactational feeding strategies to provide an adequate supply of energy and nutrients for use by the mammary gland for milk production and other mammary functions, and to stimulate growth of the lactating mammary gland.

1.6.3. Mammary gland amino acid supply

The dietary amino acid supply is often not sufficient to provide for all the requirements of the lactating mammary gland. This stimulates the animal to turn to her endogenous tissue reserves to make up the deficit and provide the additional amino acids required (see Section 1.1). This reserve is predominantly composed of skeletal muscle protein, but it is not know whether the mixture of amino acids released upon mobilization of skeletal muscle is adequate to meet the requirements of the lactating mammary gland. This amino acid mixture does not resemble the amino acid composition of myofibrillar protein from which it is derived (Table 1-4). Skeletal muscle and cardiac muscle actively catabolize several amino acids, particularly leucine, isoleucine, and valine (branched chain amino acids, BCAA), and predominantly synthesize alanine and glutamine (Young, 1970; Felig, 1975; Cheng and Goldberg, 1978). The amino acid mixture released upon mobilization of muscle protein is therefore enriched in alanine and glutamine and depleted of other amino acids, especially the BCAA.

The mammary gland has a high requirement for some essential amino acids, especially BCAA's, because there is a high concentration of essential amino acids in milk protein (Table 1-4). Also a large proportion of some essential amino acids (e.g. BCAA, phenylalanine, arginine, and threonine) are taken up by the mammary gland and apparently retained, rather than secreted in milk (Figure 1-2). Similar to the

Table 1-4 Comparison of the amino acid composition of skeletal muscle compared to the percentage uptake of amino acids by the mammary gland and their release upon mobilization of skeletal muscle protein.

	Amino acid composition, %		% of total amino acid			
	Rabbit actin[a]	**Bovine muscle[b]**	**Release from skeletal muscle[c]**	**Uptake by mammary gland[d]**	**Sow milk AA[e], %**	**Muscle release : mammary uptake[f]**
EAA						
Leu	7.0	8.5	4	9.7	8.0	--
Val	5.6	6.4	3	6.3	6.1	--
Arg	4.8	5.0	2	6.2	3.5	--
Lys	5.1	8.5	9	5.6	6.3	+
Ile	8.0	5.3	2	4.9	4.2	--
Thr	7.2	5.2	4	4.6	4.6	=
Phe	3.2	3.4	2	3.3	3.1	-
His	2.1	3.4	4	1.7	2.4	+
Met	N/R	3.0	2	1.5	1.7	=
Trp	1.1	0.8	N/R[g]	1.4	0.8	?
NEAA						
Glu + Gln	10.4	14.0	30[h]	18.8	16.8	+++
Ala	7.8	7.8	30	9.0	5.0	+++
Pro	5.1	4.5	7	8.5	13.8	-
Gly	7.5	6.5	10	6.5	5.3	+
Ser	5.9	4.6	-	5.6	6.8	?
Asp + Asn	9.0	9.3	N/R	3.4	7.5	?
Tyr	4.3	2.8	2	2.3	2.8	=
Cys	N/R	1.1	-	< 0.3	1.4	=

All amino acid data was transformed to Mol rather than g, before changing to % of total

[a] Amino acid composition of rabbit actin from skeletal muscle (from Elzinga et al., 1973).

[b] Amino acid composition of bovine longissimus muscle (from Nguyen and Zarkadas, 1989)

[c] Amino acid released from human skeletal muscle, from arterio-femoral venous differences, in the resting post-absorptive state in the hind-limb, recorded as µMol/L (Felig, 1975).

[d] Amino acid uptake by the mammary gland in the lactating pig, recorded as mMol/d (from Trottier et al., 1997).

[e] Amino acid composition of sow milk protein recorded as mMol (from King et al., 1993b).

[f] Ability of the amino acid mixture released from muscle (hind-limb) to provide the amino acid requirements of the lactating mammary gland of the pig. – denotes deficient, = denotes sufficient, and + denotes excess.

[g] A value for this amino acid was not recorded (N/R) in this experiment.

[h] The value only includes glutamine.

lactating sow, BCAA's are also retained in the mammary gland in greater quantities than they are excreted in milk in the dairy goat (Bequette et al., 1997). Thus the amino acid mixture mobilized from muscle protein does not match that required for milk biosynthesis and mammary function.

In the sow, about 25% of the essential amino acids taken up by the mammary gland are apparently retained (Trottier et al., 1997). Since the uptake of essential amino acids accounts for about 45% of the total mammary amino acid uptake, then about 12% of the total amino acids taken up by the lactating mammary gland are retained. The retained amino acids are probably used for such mammary gland functions as metabolic requirements for non-milk protein synthesis (turnover), oxidation, and metabolite formation. Some non-essential amino acids, especially glutamine, alanine, and glycine, are also retained in the lactating mammary gland of the pig (Figure 1-2). But the requirement for these amino acids is more than met by the amino acid mixture mobilized from muscle. Therefore if a sow depends upon mobilization of her endogenous protein reserves for a large proportion of her amino acid requirements,

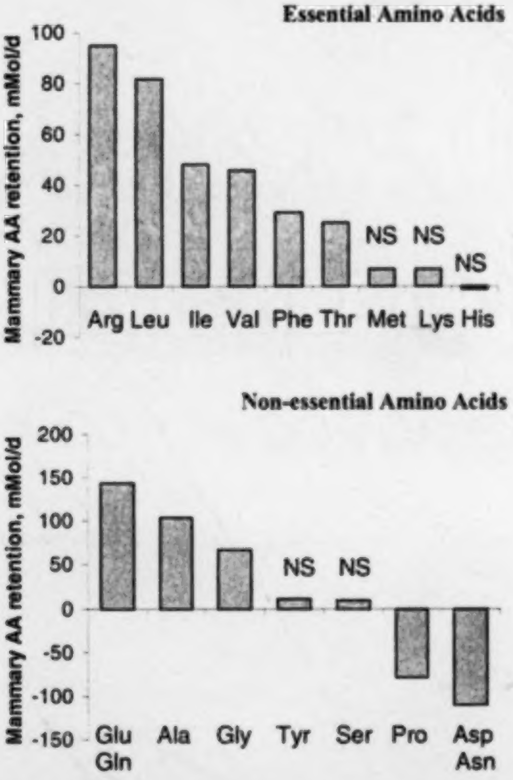

Figure 1-2 Daily amino acid retention in the mammary gland of the lactating sow. Retention calculated as daily mammary amino acid uptake – daily milk amino acid output. Adapted from Trottier et al. (1997)

the body's pool of free essential amino acids will become progressively more depleted of some essential amino acids and certain non-essential amino acids accumulate. This could act as a signal to inhibit animal performance. Certainly in the lactating pig and dairy goat, milk yield is positively related to the rate of blood flow through the mammary gland and to the arterio-venous differences of essential amino acids across the gland (Bequette et al., 1997; Trottier et al., 1997).

Essential amino acid mammary gland uptake may also be inhibited by high concentrations of non-essential amino acids that compete for use of the mammary gland transporter systems. Many amino acid transport systems are recognized in mammalian tissues, and these can be subdivided into five types that prefer:

a. **neutral amino acids**, three such systems have been identified and these prefer:
    i) small side-chains, OH or SH groups e.g. alanine, serine, and cysteine (ASC).
    ii) small unbranched side-chains e.g. alanine (A).
    iii) large branched or aromatic side-chains (L) e.g. BCAA, phenylalanine, methionine, tryptophan and tyrosine.

b. **cationic amino acids** (y^+), that prefer amino acids with positively charged side-chains e.g. lysine and arginine.

c. **anionic amino acids** (-), that prefer amino acids with negatively charged side-chains e.g. aspartate and glutamate (-).

d. **amino acids with nitrogen bearing side-chains** (N), e.g. glutamine, histidine, and asparagine.

e. **glycine** (GLY).

At least six amino acid transport pathways, based on ion dependency and substrate specificity, have been identified in the mammary gland to date (Shennan, 1998). Most of these mechanisms are located at the major site of amino acid entry into the mammary gland, the basolateral membrane of alveolar cells. However, because free amino acids are present in milk (Wu and Knabe, 1994) it is possible that such mechanisms are also present on the apical membrane (Shennan, 1998). A broad specificity Na^+-independent cationic amino acid transport system has been identified in the mammary gland of the mouse (Sharma and Kansal, 2000), rat (Shennan et al., 1994) and pig (Hurley et al., 2000). This transport mechanism interacts with both cationic and neutral amino acids and is similar to the b^{0+} and y^+L transport systems. A Na^+-independent y^+ cationic amino acid transport system has also been identified in the bovine (Baumrucker, 1984) and murine (Sharma and Kansal, 2000) mammary gland. A high-affinity, Na^+-dependent, L-like transport mechanism is responsible for uptake of valine in the porcine mammary gland, as well as diffusion (Jackson et al., 2000). A high affinity, Na^+-dependent anionic amino acid (-) transport system is present in the rat mammary gland (Millar et al., 1997; Millar and Shennan, 1999) and there is indirect evidence for the presence of a similar system in the dairy cow (Baumrucker, 1985). All the neutral amino acid transport systems (A, ASC, and L) are present in the bovine mammary gland (for review see Baumrucker, 1985). Furthermore, glutamine is transported in the rat mammary gland by two mechanisms that are likely to be a Na^+-independent, L transport system, and a Na^+-dependent N system (Calvert et al., 1998).

The presence of higher levels of some amino acids may inhibit the mammary uptake of other amino acids because of competition for uptake by the various transporter systems. Lysine uptake by porcine mammary tissue explants is strongly inhibited by physiological concentrations of arginine and ornithine and supraphysiological concentrations of leucine, methionine and alanine (Hurley et al., 2000). Similarly lysine uptake by rat mammary tissue explants was inhibited by neutral amino acids, the most notable being glutamine and alanine (Shennan et al., 1994). In order of severity methionine, alanine, glutamine, leucine, and lysine, respectively inhibited valine uptake in porcine mammary tissue explants (Jackson et al., 2000). Metabolic status may also effect mammary amino acid uptake; mouse mammary tissue explant uptake increased two-fold in the presence of arginine, and increased four-fold in the presence of the combination of insulin, cortisol and prolactin (Sharma and Kansal, 2000).

1.7 Impact of nutritional inadequacies on the reproductive axis

The reproductive axis is very sensitive to nutritional status, however the mechanism by which under-nutrition signals an impairment of reproductive function has not been elucidated. A large and growing body of evidence contradicts the 'adipostat hypothesis' of nutritional infertility (see Section 1.1.1) because the evidence for this hypothesis is correlative (Bronson and Manning, 1991; Cosgrove et al., 1991; I'Anson et al., 1991; Wade and Schneider, 1992). It was unclear how the brain could monitor the adiposity of an individual, but the discovery of leptin (adipose-derived hormone) has likely solved this issue (see Section 1.1.1). Even so there is a lack of compelling evidence linking leptin levels in non-rodent species with changes in the hypothalamic-pituitary-ovarian axis and reproductive function (see Section 1.7.6). Changes in the animal's protein (muscle) mass and loss of a critical amount of body protein could also act as nutritional modulators of the reproductive axis (see Section 1.1.1), but the mediators of this effect are as yet unknown. Acute and chronic changes to the reproductive axis can also be induced by nutritional inadequacy in the absence of changes in body composition (Britt et al., 1988; Booth 1990, Booth et al., 1994, 1996), but again the physiological basis for this remains elusive. All these factors are discussed in the following sections.

1.7.1 Nutritional mediators of fertility

The effect of under-nutrition on reproductive hormone secretion and ovulatory cycles has been extensively reviewed (Booth, 1990; Bronson and Manning, 1991; I'Anson et al., 1991; Wade and Schneider, 1992). These reviews indicate that under-nutrition affects all levels of the hypothalamic-pituitary-ovarian axis, but the gonadotropin-releasing hormone (GnRH)-secreting neurones in the hypothalamus is its primary focus; pituitary responsiveness to exogenous GnRH is not compromised in nutritionally growth-restricted lambs (Foster et al., 1989). More recently Wade et al. (1996) postulated a 'metabolic fuels hypothesis' to explain nutritionally-induced infertility which states that *"... reproductive physiology and (estrous) behaviours respond to minute-to-minute changes in the availability of oxidizable metabolic fuels"*. These authors further suggest that *"... fuel availability is probably detected in* *the caudal hind-brain and in the periphery. This information is relayed to forebrain effector neurons that control GnRH secretion and reproductive behaviours by as yet poorly defined neural pathways and neurotransmitters".*

Hypotheses linking nutrition and reproduction have evolved over time, but none suggest that body tissue reserves other than adipose tissue may be involved in signaling a nutritionally derived impairment of the hypothalamic-pituitary-ovarian axis. Although leptin does not appear to directly affect the reproductive axis in non-rodent species this hormone may be indirectly involved through its role of regulation of feed intake and energy homeostasis (see Section 1.7.7).

An investigation conducted during my M.Sc. programme provides support for the hypothesis the animal's protein status affects reproductive performance. It suggests that the increased subsequent litter size achieved by breeding first-parity sows at their second rather than first estrus after weaning may be related to the sow's protein metabolism (Clowes et al., 1994). At their first estrus after weaning young sows appeared to have a 'drive' to store maternal protein at the expense of adipose tissue depots; this was indicated by moderate peripheral insulin, and higher IGF-1 and glycerol concentrations. But assessment of the sow's metabolite and metabolic hormonal profile at the second estrus after weaning indicated that sows had replenished their maternal protein stores. It is likely these effects were implemented through changes in the animal's energy and protein status perceived at the cellular level, and alterations in the body's free amino acid pool may be involved.

Changes in the body's free amino acid pool could impact on the reproductive axis at the central (brain) level, because many amino acids have the potential to modulate reproductive status by acting directly as neurotransmitters and neuromodulators, or indirectly as precursors to CNS neurotransmitters. Tryptophan and tyrosine are respectively precursors of the neurotransmitters serotonin and the catecholamines (including dopamine, adrenaline, and noradrenaline), histamine is a precursor for the neurotransmitter histamine, and glutamate may function as a neurotransmitter itself or be metabolized to gamma aminobutyric acid (GABA) which also acts as a neurotransmitter. The plasma ratio of methionine to its competing neutral amino acids is closely associated with the influx of methionine to the brain and the concentration of its metabolite S-adenosyl methionine, which functions as a methyl donor (Rubin et al. 1974). The change in concentration of the body's free amino acid pool, and loss of a large amount of body protein may also stimulate a host of other metabolic changes which signal the reproductive axis (see Foxcroft, 1990, 1992). These may include changes in the level of hormones, such as insulin and IGF-1. These factors may act directly on the hypothalamic-pituitary-ovarian axis to alter the animal's reproductive function, and/or indirectly by moderating fuel oxidation and availability and other peripheral processes (reviewed by Schneider et al., 2000b; Ahima and Flier, 2000). Effects of these metabolic hormones at the level of the ovary are discussed in Section 1.7.3. A myriad of nutritional factors could potentially affect ovarian function because receptors for epidermal growth factor (EGF), IGF-1, insulin, GH, cortisol, and catacholamines are located on the granulosa cell, and IGF-1 receptors are found on thecal cells (I'Anson et al., 1992).

1.7.2 Central role of insulin and IGF-1

Insulin and IGF-1 have been postulated as central mediators of nutritional infertility. Increases in plasma LH levels and pulsatility and plasma insulin levels were observed after re-feeding fasted heifers (McCann and Hansel, 1986), and upon glucose infusion of nutritionally restricted gilts (Booth, 1990). Insulin is able to cross the blood-brain barrier (Woods and Porte, 1977), and concentrations of insulin in the cerebrospinal fluid reflect peripheral insulin concentrations (Tanaka et al., 2000). Insulin receptors are located on the arcuate nucleus and mediobasal hypothalamus and on the pituitary gland (Werther et al., 1987; Unger and Lange, 1997), suggesting that areas important in the control of GnRH and LH release may be responsive to insulin. Binding of insulin to its receptor in the hypothalamic region is also influenced by feed restriction (Melnyk and Martin, 1984). Intracerebroventricular injection of insulin increased LH pulsatility in the gilt, possibly by accelerating the GnRH pulse generator (Cox et al., 1989). A 5-d intracerebroventricular infusion of insulin to physiological levels also increased pulsatile LH secretion in streptozotocin-induced (chemically induced necrosis of insulin-secreting tissue) diabetic sheep (Tanaka et al., 2000). Peripheral administration of insulin to streptozotocin-induced diabetic male sheep increased pulsatile LH secretion, and this was potentiated by, but not dependent on, gonadal steroids (Bucholtz et al., 2000). However, acute intracerebroventricular administration of insulin failed to enhance LH secretion in feed restricted ovariectomized lambs (Hileman et al., 1993). Blocking the nutritionally induced increase in plasma insulin, by treatment with diazoxide, after realimentation of the 24-h fasted monkey did not prevent the increase in plasma LH (Williams et al., 1996). Thus, meal-induced insulin secretion does not provide the stimulus mediating the meal-induced increase in LH secretion. Furthermore, infusion of glucose into energy-restricted lactating sows increased peripheral insulin levels but did not increase LH secretion (Tokach et al., 1992b), suggesting that the suckling-induced suppression of the hypothalamic-pituitary axis in lactation cannot simply be overcome by increasing plasma insulin levels. Therefore, although insulin appears to affect the reproductive axis in a positive manner it is unlikely to function alone.

Insulin-like growth factor-1 has been suggested to be a longer-term nutritional mediator of reproduction, and it may act both centrally and at the ovarian level (see Section 1.7.3). Unlike plasma insulin concentrations, plasma IGF-1 concentrations do not increase rapidly (within 30 min) after realimentation of fasted gilts; an increase in plasma IGF-1 concentrations was not observed until 24 to 36 h after the gilt's feed intake was increased from maintenance to ad libitum (Booth et al., 1996). However LH pulsatility increased within 6 h of realimentation in this study. Receptors for IGF-1 are present on the anterior pituitary gland, and are almost exclusively located on cells secreting follicle-stimulating hormone (Unger and Lange, 1997), suggesting that IGF-1 may play a direct regulatory role in the pituitary gonadotropin system. Also, peripheral administration of low doses of IGF-1 to restricted-fed (50% Maintenance) castrated male sheep stimulated LH secretion.

1.7.3 Ovarian role of insulin and IGF-1

Metabolic hormones exert direct effects on the porcine ovary independent of changes in the circulating gonadotropins. Insulin treatment, irrespective of changes in gonadotropin secretion and peripheral estradiol concentration, increased the ovulation rates of cyclic gilts (Cox et al., 1987). It also reduced atresia in pre-ovulatory follicles and stimulated follicular steroid synthesis in cyclic gilts (Matamoros et al., 1990), and reduced atresia in small (< 3 mm) follicles in PMSG treated pre-pubertal gilts (Matamoros et al., 1991). Conversely, reducing peripheral insulin concentrations in PMSG treated prepubertal gilts (by treating with streptozotocin to induce diabetes) increased follicular atresia in small follicles, and reduced the ability of ≥ 3mm diameter follicles to produce estradiol, testosterone and IGF-1 compared to insulin-treated animals (Meurer et al., 1991). Withdrawal of insulin treatment from d 12 of the estrous cycle in diabetic gilts also increased follicular atresia and decreased the concentration of estradiol and IGF-1 in pre-ovulatory (≥ 7mm) follicles (Cox et al., 1994). The impaired ovarian function was observed in diabetic gilts despite the presence of adequate peripheral LH concentrations, indicating that inhibition of the ovary is not due to decreased hypothalamic-pituitary axis function. Cosgrove et al. (1992) provides more evidence that nutritional effects can act directly on the ovary. In their experiment, ovarian follicle growth occurred and estradiol synthesis increased upon realimentation of gilts, despite suppression of the expected increase in LH secretion by treatment with a synthetic progestogen (allyl trenbolone; Regumate).

Receptors for IGF-1 are located on porcine granulosa cells (Veldhuis and Furlanetto, 1985) and IGF-1 gene expression is present in the porcine ovary (Charlton et al., 1993). Therefore ovarian derived IGF-1 may mitigate the impact of acute nutritional challenges on ovarian function. Elevated follicular fluid IGF-1 and insulin levels are associated with enhanced follicular development. Dominant follicles contain higher follicular fluid IGF-1 levels in a number of species including the human (Eden et al., 1988), pig (Hammond et al., 1988), and cattle (Echterkamp et al., 1990). Follicular fluid insulin concentrations are also higher (five-fold) in follicular fluid in human follicles that contain oocytes that are fertilized (Diamond et al., 1985).

The actions of IGF-1 on the ovary include enhancement of granulosa cell steroidogenesis (Veldhuis and Furlanetto, 1985), and enhanced EGF-stimulated granulosa cell proliferation (May et al., 1988). Both IGF-1 and insulin enhanced LH-stimulated androgen production in rat ovarian thecal-interstial cells in a similar manner (Cara and Rosenfeld, 1988). Insulin and IGF-1 also enhance granulosa cell FSH-stimulated steroidogenesis (progestin and estrogen production) and LH/hCG receptor induction (May and Schomberg, 1981; Veldhuis et al., 1984; Davoren et al., 1986). But the effects of IGF-1 were far more (36 times) potent than those of insulin, suggesting that these actions were mediated through the IGF-1 receptor (Davoren et al., 1986). It is therefore not surprising that elevated ovarian follicular fluid IGF-1 concentrations in the pig are associated with enhanced follicular development and lower follicular atresia (Matamoros et al., 1991). A lower follicular IGF-1 content has also been implicated in the reduced follicular quality of medium-sized follicles in sows that lost a large amount of live-weight (-38 kg or -21% parturition live-weight) in lactation (Quesnel et al., 1998ab). These sows had potentially mobilized a large proportion of their whole-body protein mass in lactation (estimated to lose > 20% of their parturition protein mass).

The effects of IGF-1 on porcine oocyte nuclear maturation are exerted directly on the oocyte (Sirotkin et al., 1998), and these effects are likely regulated by the number and sensitivity of the IGF receptor, and the type and concentration of their binding proteins (IGFBP's). The IGFBP's are probably also associated with follicle and oocyte maturation. For example, in humans IGFBP-1 concentrations are higher in fluid from mature ovarian follicles (Kawano et al., 1997), and mRNA expression of this IGFBP increases in the liver and kidney, and potentially in the ovary, under protein deprivation and malnutrition (Takenaka et al., 1993). Follicular IGFBP's may also play an important role in IGF autocrine/paracrine regulation in the porcine ovary (Mondschein et al., 1990, 1991), and in follicular growth and differentiation, especially IGFBP-2, -4 (Zhou et al., 1996) and -3 (Whitley et al., 1998; Wandji et al., 2000). However, IGFBP-1 does not appear to be present in the porcine ovary. The IGFBP's probably exert their effects by modifying the biological activity of IGF-1 by complexing with the hormone, because the highest biological activity of IGF-1 is associated with its free form. These factors may be differentially regulated by metabolic signals, such as insulin, after weaning in sows (Whitley et al., 1998) and in untreated compared to treated diabetic pigs (Edwards et al., 1996), to allow more follicles to become available for ovulation by slowing follicular growth.

1.7.4 *Effect of lactational nutrition on the reproductive axis*

Low feed intakes and specifically low protein/lysine intakes could causes central inhibition of the reproductive axis, possibly due to loss of a proportion of the sow's protein mass and/or changes in the rate of skeletal muscle protein mobilization. This supports our previous suggestion that a decline in the sow's post-weaning reproductive performance occurs upon loss of critical amount of her body protein mass (see Section 1.1.1 and Figure 1-1a). The rate of LH pulsatility indicates the ability of the hypothalamus to secrete GnRH, and the ability of the anterior pituitary to respond to this stimulus and secretes gonadotropins. This in turn stimulates ovarian follicle development. A reduction in LH pulsatility indicates a less robust reproductive axis, likely due to reduction in the rate of GnRH release from the hypothalamus. Restrict-feeding in lactation reduced LH pulsatility in late lactation and after weaning (Zak et al., 1997a; Quesnel et al., 1998a; van den Brand et al., 2000). Lower LH pulsatility in lactation (King and Martin, 1989; Jones and Stahly, 1999b, Yang et al., 2000b) and after weaning (King and Martin, 1989) was observed in first-parity sows fed similar energy levels but low protein (~ 400 g/d) and total lysine (16 g/d) intakes in lactation. These sows likely mobilized a larger proportion of their body protein mass, as indicated by higher rates of muscle protein mobilization throughout lactation (Jones and Stahly, 1999a; Yang et al., 2000b).

The effect of lactational nutrition on ovulation rate in the pig is equivocal. A few authors observed lower ovulation rates in sows that were feed restricted in lactation (Zak et al., 1997a; van den Brand et al., 2000). Feed restriction in lactation also reduced the pre-ovulatory follicle pool size at weaning; restricted-fed sows in a 14 d (Miller, 1996) and 28 d (Quesnel et al., 1998a) lactation, respectively had fewer ≥ 3 and 4mm ovarian follicles at weaning. But the majority of researchers observed no effect of lactational feeding on ovulation rate (King and Williams 1984ab; King and Dunkin et al., 1986; Yang et al., 1989; Zak et al., 1998). However, the quality of the follicle, and the oocyte within that follicle, are important for normal oocyte maturation and early embryonic development (Ding and Foxcroft, 1994). Therefore even if a sow's ovulation rate is unaffected by nutritional deprivation the quality of the oocyte likely suffers resulting in a reduced embryo survival rate and ultimately a reduced subsequent litter size. Certainly restricted-feeding in lactation does reduce subsequent embryo survival (Hardy and Lodge, 1969; Hughes et al., 1984; Kirkwood et al., 1987; Baidoo et al., 1992; Zak et al., 1997a), indicating control of reproductive function at the ovarian level.

Measurement of the ability of follicular fluid from treated animals to support oocyte maturation of high quality generic oocytes in vitro provides a good index of the maturity of oocytes and follicles from treated sows (see Section 1.7.5). Two recent studies evaluated the effects of poor lactational nutrition on ovarian follicle and oocyte quality in first-parity weaned sows. In both studies a higher measured or estimated protein mobilization rate, at the end of lactation, could be implicated in the reduced ovarian function post-weaning, but this relationship was not explored in these manuscripts. Yang et al. (2000a) observed that follicular fluid taken from sows fed the lowest total lysine intake in lactation (16 vs > 36 g/d), about 3 d after weaning (pro-estrus), was less able support oocyte maturation. These sows had the highest fractional myofibrillar protein breakdown rate at the end of lactation (d 15) (5.6 vs < 4.2%) and therefore mobilized more protein in lactation. In the other experiment sows were either ad libitum fed in the first 21 d of lactation and restricted (50% ad libitum intake) in the last week, or restricted in the first 21 d of lactation and ad libitum fed in the last week (Zak et al., 1997b). Sows on both treatments lost a similar amount of live-weight (-29 kg) and backfat depth (-1 to –3mm). But follicular fluid, taken about 3 d after weaning, from sows that were feed restricted in the last week of lactation supported poorer generic oocyte maturation. In the last week of lactation these sows likely had a high protein mobilization rate (-18 kg live-weight and –2.8mm backfat), whereas sows on the other treatment probably mobilized very little protein over the same period (-3 kg live-weight and +3.6mm backfat). This indicates that a change in the sow's protein metabolism towards either greatly reduced protein mobilization or even protein retention enhances the environment (follicular fluid) surrounding the oocyte. This likely mediated the nutritional effects on ovarian function that could potentially result in increased embryo survival and subsequent litter size. Follicular fluid contains a complex mixture of serum proteins and factors secreted by follicular (granulosa and thecal) cells that include growth factors such as IGF-1, EGF, and TGF_β, and steroid hormones such as E_2 (Osborn and Moor, 1983; Gougeon, 1996; Driancourt and Thuel, 1998). These factors influence the ability of the oocyte to be fertilized and develop into an embryo, may be altered by nutrition, and can potentially affect embryo survival. Changes in energy balance and metabolism may also be implicated in the altered ovarian function in these sows.

1.7.5 Follicle development and maturation

Antral follicles (≥ 400 μm diameter) take 14 to 16 d to grow to ≥ 3mm diameter and be recruited into the pre-ovulatory follicle pool in the pre-pubertal gilt, and then require a further 5 d to reach the pre-ovulatory size of ≥ 8 mm (Morbeck et al., 1992). Follicles are selected into the pre-ovulatory follicle pool by follicular atresia and a concomitant block of recruitment of new follicles into this pool. Following antrum formation a follicle has the potential to be recruited, selected into the pre-ovulatory follicle pool, and subsequently ovulate in the span of one estrous cycle (d 19 to 21). If this is true for sows in lactation, and if all stages of follicular development are sensitive to nutritional modulation, nutrition in lactation and factors such as the extent of body protein loss and changes in muscle protein metabolism could negatively 'imprint' follicles at any stage of lactation. This could have lasting consequences on the ultimate size of the recruitable follicle pool at weaning, the quality of the oocytes and therefore the ovulation rate and embryo survival after weaning. Follicle quality after weaning certainly appears to be able to be 'rescued' by improved nutrition in the last week of lactation (Zak et al., 1997b; Section 1.7.4).

Zak et al. (1997b) observed that the degree to which follicular fluid is able to advance generic oocyte maturation (from pre-pubertal gilts of approximately 105 kg) reflects the ability of the oocytes within those follicles to advance their maturation in vitro. Thus, the two indices of follicle maturation (oocyte nuclear and cytoplasmic maturation) provide a good index of the maturity of oocytes and the oocyte maturing capabilities of follicular fluid. Nuclear maturation can be induced in the absence of complete cellular maturation, but both nuclear and cytoplasmic maturation is required for successful fertilization (Vanderhyden and Armstrong, 1989). Nuclear development of fully-grown oocytes is initially arrested at the diplotene stage of the first prophase (germinal vesicle stage) and is characterized by a prominent nucleus. The pre-ovulatory luteinizing hormone surge stimulates the meiotic process to proceed to the metaphase II stage where it arrests prior to ovulation. Only when the oocyte has acquired the ability to resume meiosis and complete nuclear maturation does it become capable of undergoing cytoplasmic maturation.

Cytoplasmic maturation is more difficult to measure because it involves alterations in cell metabolism, protein synthesis, membrane transport, and the localization of organelles within the cytoplasm. Cumulus cell (granulosa cells that surround the oocyte) expansion has been suggested as an index of cytoplasmic maturation (Sirard et al., 1988; Vanderhyden and Armstrong, 1989). In support of this concept, cumulus cells must expand to help control several functions of oocyte maturation including nuclear maturation rate, maintenance of oocyte penetrability to allow fertilization, and promotion of normal oocyte cytoplasmic maturation (Sirard et al., 1988; Vanderhyden and Armstrong, 1989). Further support for this concept is provided by the fact that oocytes produce factors that enable cumulus cells to 1) synthesize hyaluronic acid and undergo cumulus expansion in response to hormonal stimulation, and 2) to promote proliferation of granulosa cells (Buccione et al., 1990). These interactions in vitro do not appear to be mediated by gap junctions. Oocytes also promote the differentiation of granulosa cells into functional cumulus cells. Gap junctions between cumulus cells and the oocyte allow interactions between the somatic cells (cumulus, granulosa and thecal cells) and germ cells (reviewed by Buccione et al., 1990), nutrition may play a role (Brower and Schultz, 1982).

The ability of oocytes to maturate increases with follicle size, but only oocytes from ≥ 2.5mm diameter follicles are able to undergo complete nuclear and cytoplasmic maturation. Bovine oocytes from follicles < 2.5 mm are not capable of completing meiosis to M II (Fair et al., 1995), and those originating from follicles < 2mm cannot reach the blastocyst stage of development (Pavlok et al., 1992). The proportion of bovine oocytes that develop to the blastocyst stage increases as follicle diameter increases from 2 to 4mm to > 6mm (Pavlok et al., 1992; Lonergan et al., 1994).

1.7.6 Role of leptin in reproductive performance

The onset of puberty and maintenance of reproductive function in the adult is physiologically coupled to nutrition and energetics. Leptin appears to play a permissive role (i.e. basal levels are required) in this process in normal non-obese (Ahima et al., 1996; Cheung et al., 1997) and leptin deficient (e.g. *ob/ob* mice) rodents (Barash et al., 1996). Leptin administration reverses the effects of under-nutrition in non-obese rodents (Ahima et al., 1996; Cheung et al., 1997). It prevented the starvation-induced delay in ovulation in non-obese female mice, and increased serum LH and testosterone concentrations in fasted male mice (Ahima et al., 1996). Leptin administration also advanced puberty onset in feed restricted rats (70% ad libitum) but the onset was still ~7 d later than ad libitum fed rats (Cheung et al., 1997). Chronic leptin treatment to male and female *ob/ob* mice restored fertility as well as reduced food intake and body weight (Barash et al., 1996).

Other researchers have suggested that leptin has a positive rather than a permissive role on the reproductive axis. Treatment of adult rats with anti-leptin antiserum decreased LH pulsatility and caused all animals to remain in anestrus (Carro et al., 1997). Leptin treatment of pre-pubertal (from 21 d old) wild-type mice advanced the onset of puberty by about 2 d as assessed by vaginal opening, and 10 d as assessed by the presence of copulatory plugs (Chehab et al., 1997). The validity of the observations of Chehab et al. (1997) and even Carro et al. (1997) have been challenged by Gruaz et al. (1998). These authors suggest that the actions of leptin on the reproductive axis were confounded by leptin's ability to decrease food intake. Thus leptin may act on the reproductive axis by signaling changes in the animal's energy status, this is further described in the following section (Section 1.7.7). The leptin/NPY axis likely acts as an important link between metabolic status and mechanisms regulating appetite, growth and neuroendocrine function in the pig (Barb et al., 2000).

Although there is fairly strong evidence linking leptin to reproductive function in rodents, the evidence is not as strong for non-rodent species, including the pig. Fewer studies have examined the effects of leptin on the reproductive axis in non-rodent species, in part because of the absence of adequate methodology for measuring leptin in these species. Therefore it cannot be definitively stated that leptin plays no role in communicating the status of body reserves to the reproductive axis in non-rodent species. Central administration (intracerebroventricularly) of leptin failed to alter LH secretion in pre-pubertal gilts (Barb, 1999) or in well-fed and feed restricted (38% maintenance) ovariectomized ewe lambs (Morrison et al., 1998). However, leptin stimulated GnRH release from hypothalamic tissue in vitro (Barb et al., 2000). Central administration of leptin also failed to affect the length of lactational anovulation in lactating rats (Woodside et al., 2000). Acute effects of energy deprivation in pre-pubertal gilts that suppressed LH pulse frequency and increased serum GH concentrations failed to change serum leptin concentrations (Barb et al., 1998a). Furthermore, normal estrous cyclicity in the Syrian hamster does not require plasma leptin concentrations higher than those observed in fasted hamsters (Schneider et al., (2000a).

1.7.7. Energy expenditure, food intake, and leptin

Weigle (1994) hypothesized that "... *a peripheral signal* (probably leptin) *reflecting the total adipose tissue mass is processed by the central nervous system to augment the satiety effect of meal-related gastrointestinal satiety signals*". This author continued to conjecture that "... *with expansion of the body's fat store, an increase in the adipose-related satiety signal would lead to an increase in the adipose meal size or frequency. A reduction in this signal associated with decreasing fat stores would be followed by an increase in net food consumption*". There is very strong evidence that leptin, the adipose tissue derived hormone, is likely to be the peripheral signal that reflects adipose tissue mass (see Section 1.1.1). Although plasma leptin levels tend to increase in parallel with adipose tissue stores over the long-term, and are strongly correlated with body fat mass in normal individuals (Flier, 1997), they are not always closely regulated by fat mass and can rapidly alter due to changes in nutrition, hormones and substrates. The possible mechanisms by which leptin regulates energy homeostasis and feed intake have been extensively reviewed (Caro et al., 1996; Houseknecht et al., 1998; Barb, 1999; Ahima and Flier, 2000).

Leptin administration suppresses feed intake in the pig (Barb et al., 1998b), sheep (Morrison et al., 1998), rat (Woodside et al., 1998, 2000) and mouse (Halaas et al., 1995; Ahima et al., 1996; Cheung et al., 1997). Administration of a leptin analog (LY355101; Eli Lilly and Company) to pigs (85 to 90 kg barrows) decreased feed intake in a dose dependent manner (Wuethrich et al., 2000). Peripheral leptin levels fell within 12 h of starvation in the Syrian hamster (Schneider et al., 2000a), they decreased (-64%) after 52 h of fasting in adult humans (Boden et al., 1996) and during starvation in the mice (Ahima et al., 1996). They are also lower in lactation (a physiological condition characterized by hyperphagia) in the rat (Woodside et al., 1998, 2000) and dairy cow (Block et al., 2000) compared to pre-partum levels. The fasting-induced decrease in leptin levels was prevented by infusion of glucose to maintain glucose concentrations at basal levels, and this also maintained insulin levels (Boden et al., 1996). Peripheral leptin levels have been reported to increase within hours after a meal in rodents (Saladin et al., 1995), and increase (+300%) very rapidly (within 15 min) upon refeeding after an 18 h fast (Babo et al., 1998). In the latter study, the rapid increase in peripheral leptin was associated with a substantial (-66%) decrease in gastric leptin content within 15 min of refeeding (Babo et al., 1998). However, peripheral leptin concentrations do not increase acutely following food intake in humans (Korbonits et al., 1997), unlike insulin concentrations, suggesting that leptin is not likely to serve as a meal-related satiety signal in humans and maybe other species including the pig. Peripheral leptin concentrations also vary diurnally; serum leptin concentrations in human subjects are highest at night (midnight to early morning) and lowest in the middle of the day (noon to mid-afternoon) (Sinha et al., 1996). Serum leptin levels also increase at night in rats, but fasting prevented this diurnal variation in leptin concentrations and decreased adipose tissue leptin mRNA expression (Saladin et al., 1995). However within 4 h of refeeding, the adipose tissue leptin mRNA expression in the fasted rats was restored (Saladin et al., 1995).

Leptin's effects on feed intake and energy metabolism are probably mediated centrally. The brain centres that control feed intake and monitor the animal's energy status express mRNA for the long form of the leptin receptor (Ob-R1) in sheep (Dyer et al., 1997) and pigs (Lin et al., 2000); Ob-R1 is the only leptin receptor capable of signal transduction. Differential expression of Ob-R1 mRNA is observed in the hypothalamic region in feed-restricted and well-fed sheep (Dyer et al., 1997), suggesting that the nutritionally-mediated effects of leptin on feed intake and energy metabolism also function through alterations in leptin receptor mRNA expression. The central effects of leptin are mediated via neurotransmitters such as neuropeptide Y (NPY), proopiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), melanin-concentrating hormone (MCH), and orexins etc. (reviewed by Aubert et al., 1998; Barb, 1999; Ahima and Flier et al., 2000).

Large variations in circulating leptin concentrations occur in animals of similar adiposity (Ahima et al., 1996; Boden et al., 1996; Schneider et al., 2000ab) suggesting that factors other than fat mass may regulate leptin levels and its effects on feed intake and energy metabolism. Other tissues and organs may be involved, and skeletal muscle is a potential candidate. Both leptin mRNA and protein are expressed in this tissue (Wang et al., 1998), but at much lower level than those observed in adipose tissue; skeletal muscle leptin concentrations are 10-fold lower than those in adipose tissue. Skeletal muscle, and other tissues such as adipose tissue, the ovary, pancreas, liver and intestines also express mRNA for Ob-R1 in the pig (Lin et al., 2000).

Leptin may act as a mediator of the metabolic signal if intracellular availability of oxidizable metabolic fuels controls leptin secretion (Schneider et al., 2000b). Leptin levels change within hours of fasting or restrict feeding, and could therefore act centrally to signal changes in the animal's energy status. However, it is not known how energy intake is sensed and transduced into increased leptin expression. The hexosamine biosynthetic pathway has been suggested as a potential mechanism by which changes in the animal's metabolic status is recognized at the cellular level, and leptin synthesis and secretion appears to be influenced by the oxidative substrates that are part of this pathway. Increased expression of leptin mRNA and protein in rat skeletal muscle and adipose tissue is induced by infusion of compounds that would be expected to increase the concentrations of end-products of the hexosamine biosynthetic pathway, including glucosamine, glucose, lipid, and uridine (Wang et al., 1998). Infusion of glucosamine increased expression of leptin mRNA (+2000%) and protein (from non-detectable to detectable levels) in muscle, and leptin concentrations (+30%) in adipose tissue.

1.8 Conclusion

The young sow is frequently under profound nutritional stress in lactation. This results in excessive loss of body tissues, including adipose and protein containing tissue (predominantly muscle) in lactation. However, loss of a large proportion (over 50%) of the body's muscle mass is fatal (Newsholme and Leech, 1983), so it is likely that the sow will attempt to conserve her body reserves upon mobilization of a proportion of her muscle protein tissue. This ensures survival of the sow at the expense of the current and subsequent reproductive cycles.

Because muscle protein is the protein reserve that is predominantly mobilized (see Section 1.2) it is reasonable to measure changes in this tissue when investigating the size of the body protein loss required to reduce sow performance. The amino acid mixture released upon mobilization of the muscle protein reserves does not match that required for milk protein synthesis and mammary function (see Section 1.6.3). So if a sow is in dietary protein deficit it is likely that some free amino acids are in short supply for uptake by the mammary gland. This could limit milk biosynthesis and thus inhibit the current reproductive cycle by reducing the sow's lactational performance. Impairment of the subsequent reproductive cycle due to loss of a critical amount of body protein or alteration in protein metabolism in lactation could be a function of many factors including an inadequate supply or availability of oxidizable substrates, reduced metabolic hormones, metabolites and growth factors, and/or altered free body amino acid pool which could effect the availability of neurotransmitters (see Section 1.7.1). These factors could act at the central level (hypothalamic-pituitary axis) to impact on gonadotropin secretion, and/or at the ovarian level to impair follicle maturation and oocyte quality.

1.9 Hypotheses

The primary hypothesis of this thesis is that:

the maternal protein reserve of first parity sows at parturition is large enough to support lactation, and some degree of protein loss might be sustained over the course of lactation without loss of milk production or reproductive function. However an excessive loss of protein in lactation is associated with a decline in the sow's lactational and reproductive performance. Performance progressively declines as the degree of protein loss increases above this excessive level of protein loss.

Other hypotheses:

1. First-parity lactating sows minimize maternal N losses in lactation when dietary N is limiting, and become more efficient at utilizing the remaining dietary N.
2. First-parity lactating sows allocate additional nutrients, above those provided by ad libitum intake, towards maternal growth rather than the mammary gland.
3. Changes in muscle protein mobilization over the course of lactation are dependent on the degree by which dietary protein supply supports the requirement for substrates for milk protein synthesis. Increases in muscle protein mobilization in lactation will result in:
    - A decrease in the capacity for protein synthesis (RNA:DNA ratio) and the amount of protein per 'cell unit' (protein:DNA ratio) in muscle.
    - An increase in gene expression of components of the ATP-ubiquitin-dependent proteasome proteolytic pathway in muscle.
    - Decreases in certain free essential amino acids including the BCAA's and phenylalanine and increases in some non-essential amino acids, especially glutamine, in muscle.

4. First-parity sows that are relatively more dependent on mobilization of protein from endogenous reserves have poorer lactational and reproductive performance, regardless of the sow's parturition body mass.
5. First-parity sows with a larger initial body size at parturition will maintain lactational performance and reproductive function for a longer time if dietary protein is limiting.

1.10 Objectives

In order test these hypotheses our objectives were:

1. To determine whether sows minimize their maternal N losses in lactation when dietary N is limiting, N balance and diet digestability was measured throughout lactation in first-parity sows fed 50%, 100%, and 125% of ad libitum feed intake in lactation. These treatments established sows in three divergent metabolic states (anabolic, slightly catabolic, and grossly catabolic) in lactation.
2. Nitrogen balance and litter growth rate were used to evaluate the partitioning of N towards milk production and retention in the maternal body in first-parity sows fed to 50%, 100%, or 125% of ad libitum in lactation. Indices of skeletal muscle protein synthesis (RNA:DNA ratio) were also measured at the end of lactation.
3. To determine if sow performance declines upon loss of an excessive amount of body (muscle) protein mass, first-parity sows were fed divergent levels of protein/lysine in lactation to lose three progressively larger levels of maternal protein. Lactational performance (litter growth and milk composition) and reproductive performance (ovarian follicle variables) was measured. Sows were fed similar energy intakes so as not to incur differential losses of body fat that could confound the results.
4. To measure changes in muscle protein variables over the course of lactation a muscle biopsy procedure was developed that caused minimal disruption to the sow and piglets. Muscle samples from first-parity sows induced to lose

differential amounts of their whole-body protein mass in lactation, were collected in late gestation, mid- and late-lactation. Indices of muscle protein synthesis and degradation and changes in the muscle free amino acid pool were measured. The long head of the triceps brachii muscle was sampled because this is a typical mixed-fibre muscle in the adult pig, and can be easily accessed for muscle biopsy. This muscle contains about 70% fast-twitch glycolytic fibres (45% Type IIB, 15% Type IIA, and 10% Type IID) and 30% Type I slow-twitch oxidative muscle fibres (Uhrin and Liptaj, 1992; McAllister et al., 1997).

5. First-parity sows were fed in gestation 1) to industry standards to achieve a standard body (muscle) mass at parturition, or 2) to achieve a larger body (muscle) mass at parturition. In the subsequent lactation these sows were fed divergent levels of protein to generate two levels of body protein mobilization. To determine when a decline in animal performance occurred lactational performance (litter growth and milk composition) and reproductive performance (ovarian follicle variables) were measured. The amount of the sow's protein and fat mass mobilized was:

    - ♦ Directly measured from dissection of the carcass primal cuts (shoulder, loin and ham) into muscle, fat and bone. Based on these measures and non-invasive variables measured in real-time predictions were formulated to measure changes to the sow's muscle mass over lactation. Whole-body protein mass was also estimated from prediction equations based on live-weight and backfat depth.

    - ♦ Indirectly measured from live-weight and ultrasonic measures made sequentially, these included backfat thickness and loin muscle thickness and area. Indices of muscle protein synthesis and degradation were also measured. These included muscle protein, RNA and DNA and mRNA expression for components of the main proteolytic pathway in muscle.

- Because equations that estimate a lactating sow's muscle protein mass are not available, a final objective was to develop such equations based on dissection of the primal cuts of the carcass into muscle and fat mass.

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CHAPTER TWO

Nitrogen partitioning and skeletal muscle composition in lactating first-parity sows fed to establish three divergent metabolic states[1]

2.1 INTRODUCTION

Many modern sows generally fail to consume sufficient nitrogen and energy to meet requirements for milk production and growth during lactation (Aherne and Williams, 1992). Consequently, these sows mobilize lipid and protein reserves to make up this deficit during lactation (King et al., 1993; Everts and Dekker, 1994). Skeletal muscle is the primary source of mobilizable protein in the body (Swick and Benevenga, 1977). Whole-body protein mobilization in lactating sows can be estimated by N balance studies, and the amount of muscle protein mobilization can be estimated by changes in muscle variables (protein:DNA and RNA:DNA ratios) taken by muscle biopsy, and by changes in urinary creatinine excretion. Modifications in urinary creatinine excretion during the course of lactation reflect changes in the maternal muscle protein mass, because creatinine is synthesized in muscle and excreted in urine in amounts proportional to the animal's muscle mass (Tietz, 1986). Nitrogen partitioning was estimated by N balance studies. Dietary N is partitioned in the lactating sow towards losses in feces and urine, and retention in milk and maternal body mass. To efficiently utilize dietary N, lactating sows probably limit N losses and prioritize N toward maternal gain and(or) the mammary gland for milk synthesis.

In this experiment, we compared lactating first-parity sows fed to establish three divergent metabolic states (anabolic, slightly catabolic, and grossly catabolic) to evaluate partitioning of N towards losses and retention. We modified the 'superalimentation' model (feeding via a gastric cannula) of Matzat et al. (1990) to overcome the lactational appetite limitation and create anabolic sows during lactation. Multiparous sows were used in this experiment, and the extent of N partitioning was not quantified. Because first-parity sows have smaller appetites than multiparous sows and are still growing (accreting maternal protein), we hypothesized that first-parity lactating sows 1) allocate additional nutrients above their ad libitum intake towards maternal reserves rather than towards the mammary gland for milk production and 2) minimize N losses when dietary N is limiting.

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[1] A form of this Chapter has been previously published:

Clowes, E.J., I.H. Williams, V.E. Baracos, J.R. Pluske, A.C. Cegielski, L.J. Zak, and F.X. Aherne. 1998. Feeding lactating primiparous sows to establish three divergent metabolic states: II Effect of nitrogen partitioning and skeletal muscle composition. J. Anim. Sci. 76:1154-1164.

2.2 MATERIALS AND METHODS

This experiment was approved by the University of Alberta Animal Care Committee to ensure adherence to the Canadian Council of Animal Care Guidelines.

2.2.1 Experimental Treatments and Measurements

i) Experimental design. First-parity sows (n = 36) were offered a high-quality diet (Table 2-1) during lactation according to one of three feeding regimens designed so that sows were either made grossly catabolic (restricted-fed), slightly catabolic (ad libitum-fed) or anabolic (superalimented) by the end of a 28-d lactation. The results of the sow's reproductive performance after weaning is presented in a companion publication (Zak et al., 1998). Restricted-fed sows were made grossly catabolic in lactation by restricting their feed intake to ~3 kg/d (fed at 0600, 1330, and 2100). The expectation was that restricted-fed sows would mobilize in excess of 1 kg of live-weight/d. Ad libitum-fed sows were made slightly catabolic by offering up to seven meals between 0600 and 2100 daily, each meal was composed of 1 or 2 kg of feed. Superalimented sows were given their calculated feed allocation (~125% of their estimated ad libitum feed intake) via a stomach cannula in seven meals evenly spaced throughout the day, between 0600 and 2100. Fresh food was available to superalimented sows at all times, and alimentation of feed commenced within 3 d post-partum. The amount of feed given to superalimented sows daily was based on the results of a pilot trial conducted at the University of Alberta Research Centre. In this trial it was estimated that superalimented sows would need to receive ~125% of the estimated feed intake of the ad libitum-fed group to be in an anabolic state at the end of a 28-d lactation. Sow was considered the experimental unit and each replicate represented a 12-crate farrowing unit.

The pulsatile release of luteinizing hormone in a 12 h period just prior to (0.2 vs 2.7 and

Table 2-1 Composition of the experimental diets (as-fed basis), as a % of the diet

Ingredient	Gestation	Lactation
Barley	56.3	24.0
wheat	30.0	24.0
Soybean meal (44%)	7.0	22.0
Fishmeal	-	5.5
Sugar	-	16.0
Canola oil	-	5.0
Tallow	2.0	-
Iodized salt	0.5	-
Dicalcium phosphate	1.7	2.6
Limestone	1.4	-
Vitamin/Mineral supplement[a]	1.0	1.0
Analysis		
Digestible energy, MJ DE/kg	13.4	15.4
Crude protein, %	13.7	18.6
Lysine, %	0.56	1.05

[a] Supplied the following per kg of complete feed: 10,000 IU vitamin A, 1,000 IU vitamin D, 80 IU vitamin E, 2 mg vitamin K, 30 μg vitamin B_{12}, 12 mg riboflavin, 25 mg niacin, 25 mg calcium pantothenate, 600 mg choline chloride, 200 μg biotin, 200 mg folic acid, 5 mg ethoxyquin, 150 mg iron, 12 mg manganese, 120 mg zinc, 12 mg copper, 200 μg iodine and 100 μg selenium.

3.3 ± 0.6 pulses/12h), and after weaning (3.8 vs 6.5 and 7.8 ± 0.6 pulses/12h) were respectively lower ($P < 0.05$) in restricted-fed than ad-libitum-fed and superalimented sows. This difference in LH pulsatility was reflected in an extended wean-to-estrus interval in restricted-fed compared to ad libitum-fed and superalimented sows (6.3 vs 4.2 and 4.4 ± 0.1 d; $P < 0.05$). However, ovulation rate and embryo survival to d 30 of gestation did not differ among treatments, resulting in respectively 13.2, 12.0, and 11.3 embryos in superalimented, ad libitum-fed, and restricted-fed sows by d 30 of gestation (Zak et al., 1998).

ii) Animal management and measures. Camborough x Canabrid gilts (n = 36; Pig Improvement [Canada] Ltd., Acme, Alberta) were individually housed and fed 2 to 2.3 kg/d of a conventional gestating sow diet (Table 2-1), according to their live-weight during gestation. All feed troughs were thoroughly cleaned daily, and dry feed refusals were weighed. Between d 65 and 75 of gestation all gilts underwent surgery for the insertion of a gastric cannula (Pluske et al., 1995). On d 109 gilts were moved into individual farrowing crates and the ration was increased by 1 kg/d and changed to the lactation diet (Table 2-1). The temperature of the farrowing room was controlled at between 20 to 23°C, and an evaporative cooling system was automatically switched on if the room temperature increased above 23°C. Water was freely available to sows and piglets through nipple drinkers at all times.

Gilts were randomly and equally allocated within 36 h of parturition to one of the three nutritional treatments, restricted-fed, ad libitum-fed and superalimented. Feed intake was recorded daily by weigh-back of the food refusal (orts) from the previous day, which were dried if necessary. To facilitate direct gastric feeding of superalimented sows 0.5% xanthan gum was added to the lactation ration which was then mixed with approximately two parts water to one part feed (Pluske et al., 1995). Litter size was standardized to at least eight piglets within 2 d after parturition. Routine procedures (teeth clipping, tail docking and iron injections) were carried out 2 d post partum and male piglets were castrated between d 15 and 19 of lactation. Piglets had no access to creep feed throughout lactation. Sows and piglets were weighed and sow backfat was measured ultrasonically (Scanoprobe II, Scano, Ithaca, NY) 65mm from the midline at the last rib (P2) at farrowing and every 7 d until weaning. Nitrogen balance studies were conducted on a subset of 24 sows in early- (d 3 and 7), mid- (d 11 and 15), and late lactation (d 19 and 23). Between d 24 and 26 of lactation, a triceps muscle sample (3 to 4 g) was collected from three sows from each treatment by biopsy. The surgical technique for the muscle biopsy is described below. The tissue was immediately frozen on Dry Ice and stored at -70°C for RNA, DNA, and protein analysis. Samples of lactation diet were collected from each batch of diet and stored at -20°C for later CP, gross energy and amino acid analysis.

iii) Nitrogen balance. Sows were catheterized with an indwelling foley catheter (French size 18 to 22, 30-mL balloon type) for collection of urine. Catheters were inserted while the sows were fully conscious and standing in their farrowing crate at the time of feeding on d 2, 9 and 17 of lactation. Urine was collected over the three 5-d periods in early-, mid-, and late-lactation. Total urine was collected daily into 100 mL of 20% HCl in a plastic container, weighed, and a 1% sample by weight was composited for the three 5-day collection periods and stored at -20°C for subsequent N, creatinine, and energy analysis. Chromic oxide was incorporated into the lactation diet at a level of 0.3% and was fed to sows for at least 5 d prior to fecal collection. Fecal grab samples were collected daily throughout the collection periods, stored at -20°C, and then oven-dried at 60°C to constant weight. Fecal samples were then pooled by equal weight, within each pig and period. Feed samples were collected and stored at -20°C. Feed and fecal samples were later analyzed for N, amino acids, chromic oxide, and energy. Milk N was calculated in the three stages of lactation for the estimation of N balance using Eq. [1]:

$$\text{Milk N} = \text{Milk production} \times (\text{milk protein}/100)] \times 0.158 \qquad [1]$$

where Milk production = milk production was estimated from litter gain for this population of sows (3.88 g milk:1 g piglet gain; J. R. Pluske, personal communication), g/d; milk protein was measured in this experiment, %; and 0.158 = inverse of the conversion factor for milk protein to milk N (6.38; McDonald et al., 1988).

iv) Calculated energy balance. Energy balance (Eq. [2]) for the three stages of lactation was calculated from the measured feed energy intake and fecal and urinary energy output. The maintenance energy requirement (Eq. [3]) and daily milk energy output (Eq. [4]) of the lactating sow were calculated:

$$\text{Energy Balance} = \text{Energy intake} - (\text{Maintenance} + \text{Emilk}) \qquad [2]$$

$$\text{Maintenance} = 0.485 \times \text{Wt}^{0.75} \qquad [3]$$

$$\text{Emilk} = [(2.54 \times \text{ADG}) + (78.7 \times \text{BW}) + 153] \times (4.184 \times \text{LS})/k_l \qquad [4]$$

where Maintenance = lactating sow maintenance energy requirement, based on the mean of the values calculated by Burlacu et al. (1983) and Noblet and Etienne (1987), MJ ME/d; $\text{Wt}^{0.75}$ = sow metabolic body weight at the start of each period, $\text{kg}^{0.75}$; Emilk = energy required for milk production, kJ/d; ADG = average daily gain per piglet during the different lactational periods, g/d; BW = average piglet weight at the beginning of the given period, kg; LS = litter size; and k_l = efficiency of utilization of ME for milk production (0.72) (Noblet and Etienne, 1987).

v) Muscle biopsy. Sows were anaesthetized with Pentothal (0.17 mL 5% sodium thiopental; Sanofi Animal Health, Victoriaville, Quebec) administered via an ear vein whilst the animal was restrained with a nose snare. Anaesthesia was maintained with a close-circuit system of halothane (2%), oxygen (2.5 to 3.5 mL/min), and nitrous oxide (0.5 to 1.0 L/min), with dosage rate depending on the sow's body weight. Sows were placed in dorsal recumbency with the right fore-leg extended to expose the right shoulder. The lateral surface of the right shoulder and fore-limb was shaved and scrubbed with Betadine solution (Ayerst, St. Lauren, Quebec), swabbed with ethanol, and sprayed with Betadine solution. A skin incision, approximately 10 to 15 cm in length, was cut midway along the line between the deltoid tubercle of the humerus and the olecranon. The lateral head of the triceps brachii muscle was identified and exposed by blunt dissection along its length to within 2 cm of the olecranon and a little underneath the deltoid muscle. When a section of muscle was exposed, a strip approximately 1 cm wide and 3 to 4 g in weight was cut along its length, and immediately frozen on Dry Ice. Before closure, 0.5 to 1 mL of antibiotic ('Trivetrin', Coopers Agrofarm Inc., Ajax, Ontario, Canada) was added to the incision site. The incision was closed by three or four sutures through both sides of the cut fascia and along the cut edge of the muscle. Two or three sutures closed the connective tissue and interrupted vertical mattress sutures closed the skin. When able to walk, the sows were immediately offered food and water. Sows were monitored daily for signs of ill-health after surgery.

vi) Effect of xanthan gum on diet digestibility. The effect of addition of xanthan gum on apparent fecal N digestibility of the diet was tested using the mobile nylon bag technique (Sauer et al., 1983; de Lange et al., 1991). Approximately 1 g of the lactation diet, with or without 0.5% xanthan gum (ground through a 0.8-mm mesh screen), was added to nylon bags (25 x 40 mm, pore size 48 μm). The nylon bags were pre-digested for 2.5 h at 37°C in a pre-digestion solution (0.01N HCl and 377 IU pepsin/L in double-distilled H_2O). Three 60-kg barrows, fitted with a simple T-cannula in the duodenum, were individually housed in metabolism crates. Four nylon bags per treatment were inserted into the digestive tract, via the cannula, of these barrows in the evening and following morning of the trial. The bags were recovered in the feces 24 to 36 h later. The difference between the amount of N within the bag before and after passage through the digestive tract was used to calculate the apparent fecal digestibility of N in the diet.

2.2.2 Chemical Analyses

i) Feed, fecal and urine analysis. Prior to analysis feed and fecal samples were ground in a Wiley mill (Arthur Thomas Co., Philadelphia, PA) through a 0.8-mm screen, mixed well, and stored at 4°C until analysis. Feed and fecal nitrogen was analyzed using the FP-428 Nitrogen Determinator, System: 601-700-900 (LECO Corporation, St. Joseph, MI). Urinary nitrogen was determined on 5 g of urine using the Kjeldahl procedure (AOAC, 1980). Fecal, feed, and urine energy were determined using an adiabatic bomb calorimeter. Urine was prepared for energy determination by freeze-drying 5-mL urine samples in 10- x 5-cm plastic bags. The difference in energy value between the plastic bag and the urine was accounted for. Chromic oxide in feed and feces was determined by the method of Fenton and Fenton (1979). Amino acids in feed and fecal samples were determined by HPLC (Sedgewick et al., 1991). Methionine, cysteine, tryptophan, and proline were not determined. Creatinine was determined colorimetrically in urine, on at least 2 d of collection, using a kit (Sigma Chemical, St. Louis, MO; Catalogue No. 555) following the manufacturer's protocol.

ii) Muscle RNA, DNA, and protein analysis. Individual muscle samples were pulverized in a mortar and pestle in liquid N_2 and stored at -70°C prior to analysis. Approximately 100 mg of pulverized muscle tissue was homogenized (Polytron, Tekmar TISSUMIZER) in duplicate in 1 mL of TRIzol^{TM} (GibcoBRL/Life Technologies, Gaithersberg, MD) according to the manufacturer's protocol (Chomczynski, 1993). DNA measurements were conducted according to the fluorometric procedure detailed by Downs and Wilfinger (1983) with the following modifications. About 100 mg of pulverized muscle tissue was homogenized, in duplicate, in 1 mL of homogenization solution (1N NH_4OH and 0.2% Triton X-100). The homogenate was incubated at 37°C for 1 h, and 100 µL of this solution was diluted to 2.0 mL with assay buffer (100 mM NaCl, 10 mM EDTA, 10 mM Tris, pH 7.0) and placed on ice. Three millilitres of the fluorescent dye bisbenzimidazole (Sigma Chemical, St Louis, MO; 100 ng dye/ml in assay buffer) was added to 100 µL of the diluted homogenate on ice. The fluorescence of the sample was measured on a Perkin-Elmer Model 5050 fluorescence spectrophotometer in disposable 4.5 mL acrylic cuvettes (square four clear sides, 10 mm; Elkay Products, Inc., Shrewsbury, MA). The excitation and emission wavelengths were set at 359 and 446 nm, respectively (10-nm slit width). Calf thymus DNA (Sigma Chemicals Company, St. Louis, Missouri) was used as a standard. The reliable limit of sensitivity of this assay was 20 ng DNA. The accuracy of the estimates was determined by addition, in quadruplicate, of five quantities of DNA standard (18 to 120 ng DNA/cuvette) to aliquots of tissue homogenate. These DNA-spiked samples were parallel with the standard curve. The CV among the calculated slope values for the three assays was 4.54%. The crude tissue homogenates from the DNA analysis were assayed in duplicate, for protein using a modification of the Bradford procedure and 2 mL of Bradford dye (Darbre, 1986).

2.2.3 Statistical Analyses

All computations were performed using the GLM procedures of SAS (1990).

i) Production data. Weekly lactational feed, energy, N, and lysine intakes, and sow live-weight change and backfat changes for 26 sows were analyzed using repeated measures ANOVA. Sources of variation among sows were replicate (r = 3), treatment (t = 3), and replicate x treatment. Variation among the experimental units (sows within replicate x treatment) was used as the estimate of experimental error, and for significance testing of treatments. Significant differences among treatment x week were determined using Fisher's protected least significant difference test.

ii) Nitrogen and energy balance data. Measures of N and energy in feed, feces, and urine, and estimates in milk were used to calculate sow N and energy balances. These variables were measured in late lactation of replicate 2, and in early, mid-, and late lactation in replicate 3, except for ad libitum-fed sows in early lactation. Because of missing data two different analyses were computed, one with three treatments in mid- and late lactation only, and a second analysis with restricted-fed and superalimentation treatments across all three stages of lactation. Preliminary analyses indicated no significant contribution due to replicate, and because the majority of sows were in only one of the stages of lactation, sows were considered to be nested in treatment x stage of lactation. Therefore, the data were analyzed as treatment, stage of lactation, treatment x stage of lactation, and error.

iii) Digestibility, urinary creatinine, and muscle composition data. Feed intake was treated as a continuous variable and apparent fecal N, energy, and lysine digestibilities, and skeletal muscle RNA, DNA, protein, RNA:DNA ratio, protein:DNA ratio, and RNA:protein ratio were regressed against feed intake. Urinary creatinine in all stages of lactation, and as a percentage of levels in early lactation, was analyzed in replicate 3 only.

Variation among the experimental units (sows within replicate x treatment) was used as the estimate of experimental error, and for significance testing across treatments.

2.3 RESULTS

Of the 36 pregnant gilts allocated to treatments, 10 gilts were taken off test. Of these 10 animals, six gilts had an insufficient suckling stimulus (suckled less than eight pigs for at least 1 wk), and the remainder were removed due to illness, diarrhoea in the litter, leaking of the gastric cannula, and giving birth to four pigs. Thus 9 sows were evaluated on each of the restricted-fed and ad-libitum-fed treatments, and 8 on the superalimentation treatment. Sow production data was analyzed for the first 3 wk of lactation because sows underwent surgery for the implantation of a jugular catheter and for a muscle biopsy between d 24 and 26 of lactation. Nitrogen and energy balance data for all three stages of lactation were examined for restrict-fed and superalimented sows only because there were insufficient ad libitum-fed sows in early lactation.

Table 2-2 Nutrient intake, and weight and backfat changes in first-parity sows restricted-fed, ad libitum-fed, and superalimented in the first three weeks of lactation

	Sow feeding in lactation				Significance[b]	
	Restrict	Ad libitum	Super-aliment	SEM[a]	FL	FLxW
No. of sows	9	9	8			
Farrowing weight, kg	174	182	175	4.5	0.361	
Farrowing backfat, mm	18.3	17.9	16.8	0.81	0.357	
Feed intake, kg/d	2.96^{cx}	5.37^{y}	7.22^{z}	0.157	0.001	0.001
Digestible N, g/d	79.2^{x}	138.6^{y}	172.0^{z}	3.70	0.001	0.084
Digestible lysine, g/d	29.6^{x}	51.8^{y}	65.0^{z}	1.51	0.001	0.010
Digestible energy, MJ/d	46.8^{x}	81.8^{y}	104.5^{z}	2.36	0.001	0.001
Energy intake/maintenance, %[d]	215^{x}	346^{y}	447^{z}	12.0	0.001	0.010
Weight change, kg/wk	-9.24^{x}	-1.50^{y}	$.83^{y}$	0.217	0.001	0.129
Backfat change, mm/wk	-2.13^{x}	$-.75^{y}$	$.39^{z}$	0.283	0.001	0.724
Litter growth rate, kg/d	1.85	1.79	2.22	0.128	0.051	0.118
Litter size[e]	8.7	8.8	9.2	0.36	0.618	0.098

[a] SEM for ad libitum-fed sows. SEM for restricted-fed sows = 0.85 x SEM for ad libitum-fed sows, SEM for superalimented sows = 0.91 x SEM for ad libitum-fed sows.

[b] Effects: FL, feed level in lactation; FL x W, interaction between feed level and week of lactation.

[c] Least square mean.

[d] Maintenance energy requirement = 0.485 x $wt^{0.75}$, MJ DE/d, with $wt^{0.75}$ calculated based on animal weight at the start of each week, kg.

[e] Average litter size in the first 3 wk of lactation.

[xyz] Means within a row lacking a common superscript letter differ by value indicated.

Table 2-3 Nitrogen partitioning in first-parity sows restricted-fed, ad libitum-fed, and superalimented in the first three weeks of lactation

	Sow feeding in lactation				Significance[b]		
	Restrict	Ad libitum	Super-aliment	SEM[a]	FL	S	FLxS
N intake, g/d	103.4^{cx}	172.5^{y}	252.0^{z}	5.99	0.001	0.062	0.367
Fecal N, g/d	10.9^{x}	24.5^{y}	57.2^{z}	1.82	0.001	0.001	0.003
Urinary N, g/d	43.1^{x}	79.1^{y}	94.5^{z}	3.83	0.001	0.001	0.032
N retention, g/d[d]	49.3^{x}	68.9^{y}	100.2^{z}	5.84	0.001	0.001	0.116
Milk N, g/d[e]	72.3	76.3	77.5	3.58	0.633	0.370	0.470
N balance, g/d	-23.0^{x}	-7.4^{x}	22.8^{y}	5.87	0.001	0.001	0.484
Urinary creatinine, g/d	6.31	8.25	8.04	.61	0.147	0.955	0.410
Protein digestibility, %[f]	89.5^{x}	85.8^{y}	77.3^{z}	.84	0.001	0.001	0.088
Fecal N/N intake, %	10.5^{x}	14.2^{y}	22.7^{z}	.85	0.001	0.001	0.088
Urinary N/digestible N, %	46.6	54.5	48.4	2.46	0.098	0.001	0.239
Urinary Creatinine, %[g]	78^{x}	93^{xy}	104^{y}	6.0	0.038	0.552	0.725
Milk N/digestible N, %	78.3^{x}	52.7^{y}	40.1^{z}	3.67	0.001	0.285	0.632
Milk N/retained N, %	150.9^{x}	126.4^{x}	80.6^{y}	9.64	0.001	0.001	0.557

[a] SEM for ad libitum-fed sows. SEM for restricted-fed sows = 1.10 x SEM for ad libitum-fed sows, SEM for superalimented sows = 1.18 x SEM for ad libitum-fed sows.

[b] Effects: FL, feed level in lactation; S, stage of lactation; FL x S, interaction between feed level and stage of lactation.

[c] Least square mean

[d] N retention = N intake - (fecal N + urinary N).

[e] Milk N (g/d)= [(3.88 x daily litter gain) x (protein % in milk /100)] x 0.158, where % milk protein = milk protein content in the experiment (Pluske et al., 1998)

[f] Apparent fecal digestibility, %.

[G] Urinary creatinine, as a percentage of values in early lactation, %.

[xyz] Means within a row lacking a common superscript letter differ by value indicated.

2.3.1 Treatment Effects

There was no difference in sow weight and backfat thickness at farrowing among the three treatments (Table 2-2). Ad libitum-fed sows ate intermediate levels of digestible N, lysine, and energy (Table 2-2) and excretion of N in urine and feces was intermediate for ad libitum-fed sows compared with the other two treatments (Table 2-3). Ad libitum-fed sows lost both weight (-4.5 kg) and backfat (-2.3 mm) during lactation and were in negative energy balance in mid-lactation (-12.1 ± 6.4 MJ ME/d) but close to zero energy balance in late lactation (-1.1 ± 5.2 MJ ME/d). They were also in negative N balance in mid-lactation (-26.2 ± 9.1 g/d) but were in positive N balance in late lactation (11.3 ± 7.42 g/d).

Restricted-fed sows consumed 57% of the digestible N, lysine, and energy intake of ad libitum-fed sows. This level of intake supplied over twice the sow's calculated

Table 2-4 Calculated energy balance (MJ ME/d) in first-parity sows restricted-fed, ad libitum-fed, and superalimented in the first three weeks of lactation

	Sow feeding in lactation				Significance[b]		
	Restrict	Ad libitum	Super-aliment	SEM[a]	FL	S	FLxS
Energy intake, MJ ME/d[c]	50.5^{dx}	82.0^{y}	112.4^{z}	2.54	0.001	0.001	0.449
Maintenance, MJ ME/d[ef]	20.5^{x}	22.3^{y}	22.5^{y}	0.34	0.003	0.005	0.411
Milk energy, MJ ME/d[g]	62.7	66.3	66.1	2.77	0.374	0.202	0.766
Energy bal, MJ ME/d[h]	-32.7^{x}	-6.6^{y}	20.9^{z}	4.13	0.001	0.109	0.952
Energy dig, %[i]	88.3^{x}	85.9^{y}	80.2^{z}	.49	0.001	0.002	0.030

[a] SEM for ad libitum-fed sows. SEM for restricted-fed sows = 1.10 x SEM for ad libitum-fed sows, SEM for superalimented sows = 1.18 x SEM for ad libitum-fed sows.

[b] Effects: FL, feed level in lactation; S, stage of lactation; FL x S, interaction between feed level and stage of lactation.

[c] Energy Intake (MJ ME/d) = GE intake - (fecal energy + urinary energy)

[d] Least square mean.

[e] Maintenance energy requirement (MJ DE/d) = $0.485 \times wt^{0.75}$

[f] Calculated for each period of lactation based on animal weight at the start of each period, kg.

[g] Milk energy = $([(2.54 \times ADG) + (78.7 \times BW) + 153] \times 4.184 \times \text{litter size})/k_l$ MJ ME/d, where ADG = ADG per pig during the period of lactation, g; BW = weight of the average pig at the beginning of the short period, kg; k_l = efficiency of utilization of ME for milk production (0.72) (Noblet et al., 1987).

[h] Energy Balance (MJ ME/d) = Energy intake - (Maintenance energy requirement + milk energy)

[i] Apparent fecal digestibility (%).

[xyz] Means within a row lacking a common superscript letter differ by value indicated.

maintenance energy requirement (Table 2-2). Restricted-fed sows lost more ($P < 0.001$) weight and backfat during lactation, excreted less ($P < 0.001$) fecal and urinary N, but secreted quantities of milk N similar ($P = 0.63$) as those secreted by sows on the two higher intake levels (Table 2-3). Consequently, restricted-fed sows utilized a greater percentage of their digestible N for milk production ($P < 0.001$), resulting in a greater loss of maternal N compared to ad libitum-fed sows. Energy intake and energy balance were also lower ($P < 0.001$) in restricted-fed sows, but there was no difference in milk energy secreted among the three treatments (Table 2-4).

Superalimented sows consumed between 124 and 128% of the digestible N, lysine, and energy intakes of ad libitum-fed sows. This level of intake supplied nearly 4.5 times the sow's calculated maintenance energy requirements (Table 2-2). Superalimented sows gained weight (+2.5 kg) and backfat (+1.2 mm) during lactation (Table 2-2). They secreted similar quantities of milk N, and hence utilized a smaller percentage of their digestible N for milk production than sows fed the lower intake levels (Table 2-3). Overall, superalimented sows were in positive N and energy balance during lactation

(Tables 2-3 and 2-4). In early (-4.1 ± 16.9 g/d) and mid-lactation (-1.9 ± 12.6 g/d), superalimented sows were in close to zero N balance but by late lactation (+42 ± 9.8 g/d) they were in positive N balance.

2.3.2 Diet digestibility

From the digestibility trial, using the nylon bag technique, the addition of 0.5% xanthan gum to the diet had no effect on apparent N digestibility (90.3 ± 3.49 vs 91.5 ± 3.66% for diets containing and not containing xanthan gum, respectively). Apparent fecal digestibilities for protein, energy, and all amino acids, except histidine, were higher ($P \leq 0.001$) in restricted-fed than ad libitum-fed sows, and higher in ad libitum-fed than superalimented sows (Table 2-3, 2-4 and 2-5). The apparent fecal digestibilities of protein

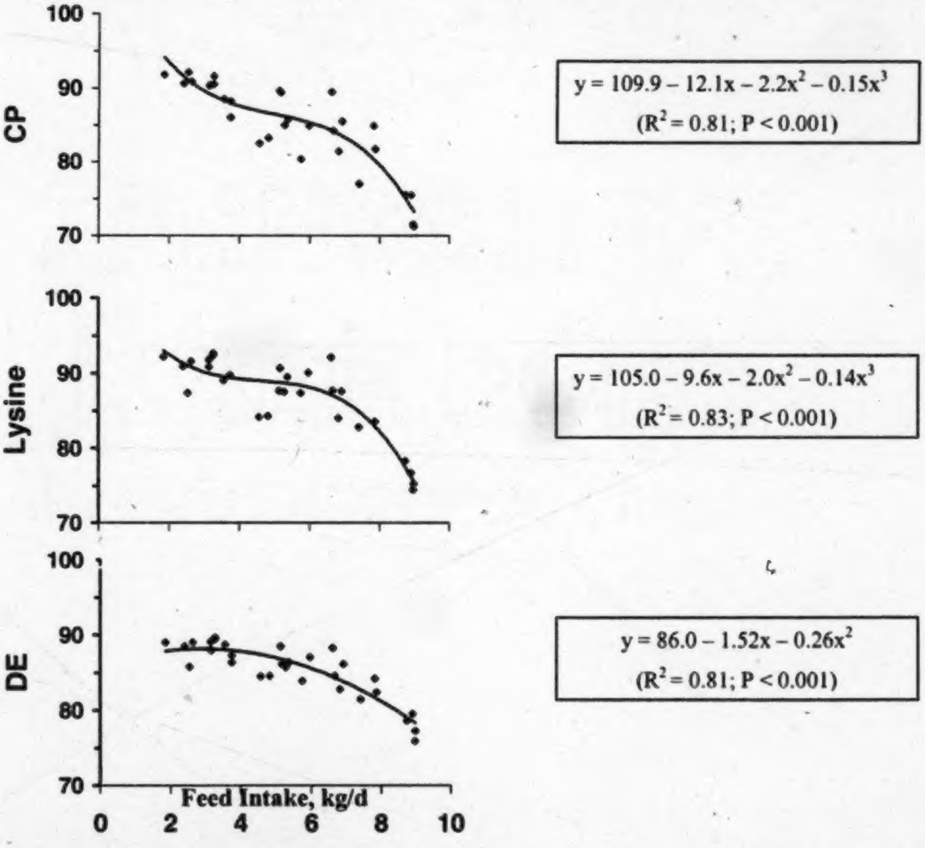

Figure 2-1 Effect of feed intake on apparent fecal digestibility (%) of crude protein, lysine, and energy in lactating first-parity sows

Table 2-5 Apparent fecal digestibility of selected dispensable and indispensable amino acids in first-parity sows restricted-fed, ad libitum-fed, and superalimented in the first three weeks of lactation

	Sow feeding in lactation				Significance[b]		
Amino Acid	Restrict	Ad libitum	Super-aliment	SEM[a]	FL	S	FL x S
Indispensable							
Histidine	94.3^{cx}	92.9^{x}	88.5^{y}	0.60	0.001	0.190	0.385
Threonine	88.2^{x}	84.7^{y}	77.3^{z}	1.18	0.001	0.224	0.200
Phenylalanine	91.8^{x}	88.8^{y}	82.9^{z}	0.72	0.001	0.046	0.196
Tyrosine	88.8^{x}	84.7^{y}	77.0^{z}	1.10	0.001	0.095	0.115
Isoleucine	90.1^{x}	86.1^{y}	78.3^{z}	0.90	0.001	0.045	0.217
Leucine	91.2^{x}	87.7^{y}	81.1^{z}	0.75	0.001	0.040	0.118
Valine	90.2^{x}	86.4^{y}	78.8^{z}	0.86	0.001	0.031	0.110
Arginine	94.5^{x}	92.5^{y}	87.6^{z}	0.49	0.001	0.011	0.022
Lysine	91.0^{x}	87.6^{y}	79.7^{z}	0.89	0.001	0.045	0.073
Dispensable							
Alanine	87.0^{x}	81.7^{y}	71.6^{z}	1.07	0.001	0.011	0.025
Aspartate	90.7^{x}	87.5^{y}	79.9^{z}	0.82	0.001	0.039	0.127
Glutamate	94.8^{x}	92.1^{y}	87.1^{z}	0.57	0.001	0.077	0.207
Glycine	90.8^{x}	87.3^{y}	80.6^{z}	0.90	0.001	0.089	0.074
Serine	92.3^{x}	89.8^{y}	84.8^{z}	0.58	0.001	0.033	0.041

[a] SEM for ad libitum-fed sows. SEM for restricted-fed sows = 0.83 x SEM for ad libitum-fed sows, SEM for superalimented sows = 1.11 x SEM for ad libitum-fed sows.

[b] Effects: FL, feed level in lactation; S, stage of lactation; FL x S, interaction between feed level and stage of lactation.

[c] Least square means

[xyz]Means within a row lacking a common superscript letter differ by value indicated.

and lysine decreased ($P < 0.001$) in a cubic manner with increasing feed intake, and the apparent fecal digestibility of energy decreased in a quadratic fashion with increasing feed intake (Figure 2-1).

2.3.3 *Stage of Lactation*

Nitrogen intake and N excreted in feces increased between mid- and late lactation, but N excreted in urine decreased with stage of lactation (Table 2-6). The percentage of retained N secreted as milk also decreased ($P < 0.001$) with stage of lactation. Similar effects of stage of lactation were seen when comparing all three stages of lactation in restricted-fed and superalimented sows only. Milk N increased ($P < 0.05$) between early (57 ± 7.0 g/d) and mid-lactation (79 ± 5.0 g/d), but it did not differ between mid- and late lactation (70 ± 4.6 g/d). Urinary N as a percentage of digestible N decreased ($P < 0.08$) between early (72.3 ± 8.54%) and mid-lactation (51.1 ± 6.86%), but there was no difference between mid- and late lactation (42.7 ± 5.69%). Nitrogen retention increased as lactation

Table 2-6 Nitrogen partitioning in mid-lactation (d 11-15) and late lactation (d 19-23) in first-parity sows fed divergent levels of intake in the first three weeks of lactation

	Stage of Lactation			
	Mid (d 11-15)	Late (d 19-23)	SEM[a]	Significance
N intake, g/d	168.4[b]	183.0	5.65	0.062
Fecal N, g/d	25.1	36.7	1.71	0.001
Urinary N, g/d	84.0	60.5	3.61	0.001
N retention, g/d[c]	59.3	86.3	5.00	0.001
Milk N, g/d	77.4	73.3	3.37	0.370
N balance, g/d	-18.1	13.0	5.53	0.001
Protein digestibility, %[d]	86.2	82.2	0.80	0.001
Energy digestibility, %[d]	85.9	83.7	0.47	0.002
Fecal N/N intake, %	13.8	17.8	0.80	0.001
Urinary N/digestible N, %	57.9	40.9	2.32	0.001
Milk N/digestible N, %	59.6	54.5	3.46	0.285
Milk N/retained N, %	142.8	95.8	9.08	0.001

[a] SEM for Period 2, SEM for Period 3 = 0.89 x SEM for Period 2.
[b] Least square mean.
[c] N retention = N intake - (fecal N + urinary N).
[d] Apparent fecal digestibility (%).

progressed for all treatments; however restricted-fed sows did not achieve a positive N balance at any time.

2.3.4 Skeletal muscle RNA, DNA, and protein and urinary creatinine excretion

Sows recovered quickly from the muscle biopsy surgery and, after 24 h of minor stiffness, were not hindered by the incision site. Treatment differences in skeletal muscle variables were observed (Figure 2-2). This confirmed that the lateral head of the triceps is a good site for observing changes in the skeletal musculature of the lactating sow. In late lactation (d 24 to 26), skeletal muscle RNA content and RNA:DNA ratio increased ($P < 0.05$) in a curvilinear fashion with increasing feed intake, reaching a plateau at intakes seen in ad libitum-fed and superalimented sows (Figure 2-2a and b). Skeletal muscle DNA content decreased linearly ($P < 0.10$) with feed intake, and the protein:DNA ratio increased linearly ($P < 0.001$) with increasing feed intake (Figure 2-2c and d). Excretion of urinary creatinine, as a percentage of creatinine in early lactation, was lower ($P < 0.05$) in restricted-fed than superalimented sows and intermediate for the ad libitum-fed sows (Table 2-3).

2.4 DISCUSSION

When first-parity sows were superalimented during lactation, they partitioned the extra nutrients they received almost exclusively into their own bodies and not into milk production; superalimented and ad libitum-fed sows produced the same estimated amount of milk energy (66 MJ/d) and milk N (76 g/d). Superalimented sows retained an additional 31 g/d of nitrogen over and above the sows fed ad libitum, and almost all of this (96%) appeared in maternal protein. This supports the hypothesis that first-parity sows allocate any additional nutrients towards their own maternal reserves. It is in contrast to the multiparous sows of Matzat et al. (1990) that, when superalimented to 117% of their ad libitum intake, channeled extra nutrients into maternal tissue accretion and also into milk production. Why the difference between first-parity and multiparous sows?

A simple explanation for this is that the first-parity sows in our experiment were younger and physiologically less mature than the multiparous sows used by Matzat et al. (1990). Everts (1994) suggested that sows have a biological 'need' to achieve a pre-determined protein body mass (approximately 35 kg) during their lifetime, and the closer an animal is to this protein mass the smaller the 'drive' to achieve this mass. Therefore, first-parity sows have a greater 'drive' to grow (accrete maternal protein) during lactation than more mature animals of a larger maternal protein mass. In contrast, multiparous sows are closer to their mature protein mass, have a smaller 'drive' to grow, and therefore partitioned more nutrients towards the mammary gland for milk production.

If accretion of maternal protein has priority over milk production in first-parity sows, then it follows that as food intake is restricted, maternal growth should have priority over milk production, and milk production in turn should fall. This was not the case in the current experiment. When sows were severely restricted in food intake, they extensively mobilized their protein reserves in order to maintain their milk production, which did not differ among the three treatments for the majority of lactation (Pluske et al., 1998); restricted-fed sows mobilized 20 g N/d more than ad libitum-fed sows.

From our N balance studies, we estimated that restricted-fed sows mobilized 3.6 kg protein in the first 3 wk of lactation. This value is in agreement with the predicted loss of

3.9 kg of maternal protein from restricted-fed sows, calculated using the equation of Whittemore and Yang (1989). From this equation it was also calculated that by d 14 and 21 of lactation restricted-fed sows had lost respectively approximately 11 and 14% of their parturition protein mass, whereas the other two treatments had either lost a very small proportion (< 2%) of their protein mass or had accreted protein. The physiological implications of this maternal protein loss were evident by the end of lactation as restricted-fed sows showed reduced litter gains and reduced reproductive performance.

Piglet growth rate in wk 3 and 4 of lactation were respectively more than 10 and 20% lower ($P < 0.001$) in restricted-fed than ad libitum-fed sows (Pluske et al., 1998). By

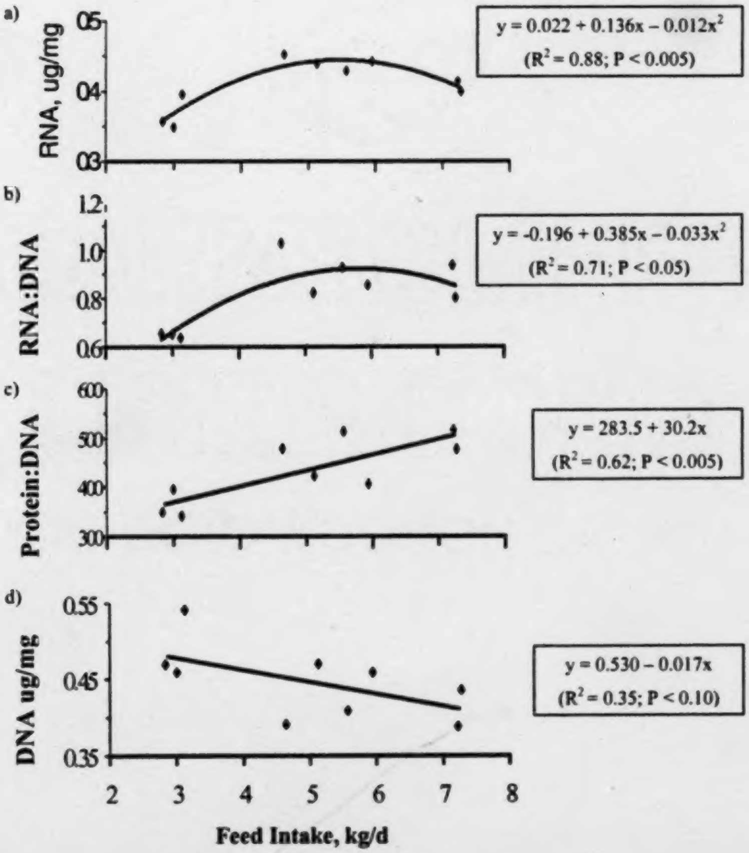

Figure 2-2 Effect of feed intake on skeletal muscle a) RNA concentration, b) RNA:DNA ratio, c) protein:DNA ratio, and d) DNA concentration at the end of lactation in first-parity sows

feeding protein-inadequate diets during lactation to pigs (Mahan and Mangan, 1975; Verstegen et al., 1985; Kusina et al., 1999) and rats (Friggens et al, 1993), animals lost a large degree of body protein and showed a reduced litter growth rate by mid- to late-lactation.

Up to 25 to 30% of maternal protein can be mobilized by lactating dairy cows (Botts et al., 1979), rats (Pine et al., 1994a), and sows (Kotarbinska, 1983; Mullan and Williams, 1990). Lactational performance was reduced in the dairy cow when more than half this protein reserve (10 to 15% of maternal protein) had been mobilized (Botts et al., 1979). The reduction in piglet growth in restricted-fed sows upon loss of a calculated ~11 to 14% of their body protein also agrees with this degree of loss of protein reserve. Assuming that excretion of urinary creatinine is proportional to the animal's muscle mass (Tietz, 1986), the reduction (-24%) in urinary creatinine observed in restricted-fed sows in late lactation indicates that a similar amount of skeletal muscle protein was mobilized from these sows during lactation. This value agrees with the estimation (25 to 30% of muscle) based on the calculated protein loss of such sows, assuming muscle is the main source of mobilizable protein (Swick and Benevenga, 1977) and accounts for about 45% of the protein in the body (Young, 1970). However, we cannot state unequivocally that the high degree of protein loss achieved by restricted-fed sows is the main factor affecting litter and reproductive performance. Restricted-fed sows also lost a high degree of backfat compared to the ad libitum-fed and superalimented sows (-8.9, vs -3.7, and +1.8mm). Therefore experiments designed in which lactating sows lose divergent levels of body protein but similar and small degrees of body fat need to be conducted to answer that question.

Milk production is a function of mammary gland nutrient uptake and biosynthetic capacity. These are determined by a number of factors including precursor availability and uptake by the mammary gland (Boyd et al., 1995). In this experiment, we increased first-parity sow nutrient intake during lactation to the level that sows accreted maternal tissues without increasing milk production. Thus, even though the growth potential of the suckling pig is greater than observed in this experiment (Boyd et al. 1995; Williams, 1995) superalimented sows did not mobilize their protein reserves and partition additional nutrients towards the mammary gland to increase milk production. This suggests that sow milk production limits litter growth and that other factors, such as endocrine control, may suppress milk production.

2.4.1 Skeletal Muscle

Skeletal muscle is the main source of mobilizable protein (Swick and Benevenga, 1977). Therefore, changes in skeletal muscle composition, and also in N balance, reflect changes in whole-body protein mobilization. In turn, changes in urinary creatinine excretion reflect changes in maternal muscle mass. Together, the N balance studies, percentage changes in urinary creatinine excretion, and the observed changes in skeletal muscle composition indicated that sows provided with increasing levels of nutrient intake mobilized progressively less of their maternal protein reserves and, when food intake was high enough, even accreted protein during lactation. The lower skeletal muscle protein:DNA ratio (amount of protein per cell unit) observed in restricted-fed sows is indicative of a reduction in cell "size" and protein stores. Similarly, Brendemuhl et al. (1989) observed that first-parity sows fed low protein levels (61 g N/d) during lactation mobilized more protein from their shoulder muscle (left supraspinatus muscle) than sows fed twice this amount.

Both lactating women (Motil et al., 1990) and pigs use adaptive mechanisms to promote conservation of skeletal muscle protein stores. By relating tissue RNA (indicator of capacity for protein synthesis) to DNA concentration the protein synthetic capacity "per muscle cell unit" can be estimated. Muscle DNA concentrations can be used as an index of cell number in the lactating sow, even though these cells are multinuclear, because porcine muscle DNA concentrations remain fairly constant after 5 mo of age (Powell and Aberle, 1975). Lower skeletal muscle RNA:DNA ratios in restricted-fed lactating sows, compared with sows fed higher intakes, suggests that a reduction in the rate of muscle protein synthesis is one of the mechanisms of protein conservation in such sows. This is supported by data showing reduced absolute rates of muscle protein synthesis in lactating dairy goats (Champredon et al., 1990; Baracos et al., 1991) and rats (Pine et al., 1994b). Because ad libitum-fed and superalimented sows were in zero or positive N balance in late lactation, no N-conserving mechanisms would have been implemented in these animals. This was reflected in their RNA:DNA ratios, which were greater than those of restricted-fed sows, suggesting that the protein synthetic capacity in muscle was maximal in these animals at the end of lactation. This difference among treatments in late lactation may explain why plasma IGF-1 levels 12 h prior to weaning did not differ between superalimented and ad libitum-fed animals (53.2 and 51.2 ± 1.8 ng/mL) but were lower ($P < 0.01$) in restricted-fed (25.5 ± 1.0 ng/mL) animals (Zak et al., 1998).

2.4.2 Stage of lactation

Similar to the observations of Noblet and Etienne (1987), as lactation progressed all sows in this experiment adapted to the increasing requirement for N in milk by excreting less N in urine, reflecting a decreased hepatic amino acid metabolism. This was especially true for restricted-fed sows that were fed an extremely limiting intake during lactation yet maintained their milk production at a level similar to that maintained by ad libitum-fed sows for the majority of lactation. The deficit in nutrient requirements for milk production was supplied by extensive mobilization of maternal protein and lipid reserves.

2.4.3 Diet Digestibility

The reduction in apparent digestibility with increase in feed intake observed in our experiment agrees with the findings of Parker and Clawson (1967). In their study, increasing the feed intake of multiparous, lactating sows from 2.7 to 8.1 kg/d decreased the coefficient of apparent DM digestibility from 89 to 85% due to an increase in the rate of passage of ingesta through the gastrointestinal tract. But, despite the reduced apparent digestibility, the intake of digestible N, lysine, and energy by superalimented sows was still greater than that in the other treatments. The quadratic relationship between protein digestibility and feed intake suggests that when sows were fed more than their voluntary feed intake (approximately 5 to 6 kg/d) apparent digestibility decreased. Thus, the gastrointestinal tract of lactating sows may not be capable of efficiently digesting feed after intake exceeds the sow's voluntary intake. The additional feed probably increased peristalsis, and therefore passage rate of feed through the gastrointestinal tract. This would result in less time being available for enzymes to digest the food and cause the reduction in apparent fecal digestibility observed.

2.4.4 Conclusion

First-parity sows prioritize additional nutrients administered during lactation towards their maternal protein reserves rather than the mammary gland. This suggests that first-parity sows limit the nutrient uptake of suckling pigs. We saw no increase in milk production by administering feed intakes considerably above ad libitum intake to young sows. However, if the piglets had provided a larger nutrient drain (i.e. larger litter size and greater suckling stimulus) on the sow, partitioning of N to the mammary gland may have increased in superalimented compared to ad libitum-fed animals. Furthermore, when N is limiting during lactation, sows mobilize their protein reserves and may even implement adaptive mechanisms, such as a reduction in muscle protein synthesis, to conserve maternal protein stores.

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CHAPTER THREE

Selective protein loss in lactation is associated with reduced litter growth and ovarian function

3.1 INTRODUCTION

Sows often lose large amounts of adipose and protein-containing tissue in lactation as a result of their high milk yields and relatively small appetites (Aherne and Williams, 1992; Dourmad et al., 1998; Sauber et al., 1998). Live-weight losses in excess of 20 kg, or 10 to 15% of their body weight, in lactation are associated with reduced milk production (King and Dunkin, 1985; Verstegen et al., 1985) and lower subsequent reproductive performance (Aherne and Kirkwood, 1985; Prunier et al., 1993). However it is unclear whether these negative effects on sow performance are attributable to depletion of maternal fat reserves, protein reserves, or both.

Excessive fat loss in lactation has been implicated in reduced reproductive performance in the weaned sow (Yang et al., 1989). The 'adipostat hypothesis' suggests that circulating concentrations of hormones, such as leptin, reflect levels of body fatness and act as a signal to control food intake and reproduction (Houseknecht et al., 1998; Keisler et al., 1999; Schneider et al., 2000b). Leptin is a potent regulator of reproduction in the rodent (Ahima et al., 1996; Chehab et al., 1997; Cheung et al., 1997). However, in other species, including large domestic species such as the pig, although the effects of leptin cannot be discounted other mechanisms such as availability, uptake and oxidation of metabolic fuels are likely the primary factors that affect the reproductive axis (Booth, 1990; Bronson and Manning, 1991; I'Anson et al., 1991; Wade and Schneider, 1992; Schneider et al., 2000b). Leptin, insulin, IGF-1 and other hormones probably affect the reproductive axis indirectly by moderating metabolic fuel oxidation and availability, and other peripheral processes (Ahima and Flier, 2000; Schneider et al., 2000a). Evidence for leptin's potential effects on peripheral processes is supported by the expression of mRNA for the leptin receptor (long form) in the brain, and in skeletal muscle, adipose tissue, and the ovary, pancreas, liver and intestine in the pig (Lin et al., 2000).

Several researchers have suggested that loss of protein (muscle) during lactation may be a more important factor affecting subsequent reproductive performance than loss of adipose tissue (King, 1987; Britt et al., 1988). High levels of protein depletion are associated in the pig with extension of the wean-to-estrus interval (Jones and Stahly, 1999b), reduction in LH pulsatility in lactation and upon weaning (Jones and Stahly, 1999b; King and Martin, 1989; Yang et al., 2000b), and a reduced ovarian function (Yang et al., 2000a). High levels of protein depletion are also associated with a reduction in milk production (Kusina et al., 1999) and litter growth rates in lactation (Mahan and Mangan, 1975; King and Dunkin, 1985; Kusina et al., 1999; Jones and Stahly, 1999a). From a review of published data we established that the subsequent wean-to-estrus interval in first parity sows is positively related to the estimated whole-body protein loss as a percentage of body protein at parturition, and these relationships appear to be curvilinear above a 10% loss of body protein (Chapter 1).

While there appears to be some association between a decline in reproductive and lactational performance and body protein loss, it has not been resolved whether protein loss per se (as opposed to concurrent fat loss) might be related to these changes. To test this it would be necessary to design an experiment where protein loss was elicited independently of fat loss. It may be hypothesized that maternal protein reserves at first parity are sufficient to support lactation, and that some degree of protein loss might be sustained over the course of early lactation without any functional loss in milk biosynthesis or reproductive functions.

In order to test this hypothesis we fed first-parity sows divergent levels of protein/lysine in lactation to achieve three progressively greater levels of maternal protein loss. Sows were fed similar energy intakes so as not to incur differential losses of body fat that could confound our results. Lactational performance was determined over the course of lactation by litter growth and milk composition. Effects on the reproductive axis were measured as differences in the ovarian follicle population, follicular fluid estradiol (E_2) and IGF-1 concentrations, and the ability of follicular fluid from treated sows to support nuclear and cytoplasmic maturation of generic oocytes. These changes were related to the degree of body protein loss calculated according to the equations of Whittemore and Yang (1989).

3.2 MATERIALS AND METHODS

This experiment was conducted in accordance with the Canadian Council of Animal Care Guidelines and was approved locally by the Institutional Animal Policy and Welfare Committee.

3.2.1 Experimental Treatments and Measurements

Thirty-six Camborough x Canabrid gilts (PIC, Acme, Alberta) were allocated to the study. Gilts were bred on at least their second estrus, and upon breeding were housed in an environmentally controlled room, in groups of five to seven animals. Gilts were individually fed 2.5 kg/d of a conventional dry sow diet (Table 1) once daily for the first 30 d of gestation. There was some heterogeneity in the initial live-weights of the available animals (96 to 143 kg) due to age differences (168 to 261 d). Therefore, to achieve a similar target live-weight (~195 kg) and backfat depth (~17 mm) at parturition gilts were fed, either 2.8, 2.5 or 2.3 kg/d from d 30 of gestation, based on live-weight. From d 109 of gestation until farrowing gilts were offered an additional 0.5 kg of feed/d and were moved into individual farrowing crates in an environmentally controlled room containing 12 crates. Sows were randomly and equally allocated, at parturition, to be fed a mash diet formulated to provide three divergent protein and lysine, but similar energy intakes (Table 3-1) to achieve either a low, moderate or high amount of protein loss in lactation. To reduce the variance in feed intake between animals, feed was offered three times daily, at approximately 85% of the ad libitum intake of lactating first-parity sows in our herd. Feed disappearance was measured daily by weigh-back of feed refusals. Water was freely available to sows and pigs through nipple drinkers throughout lactation.

Sow backfat depth (65mm from the midline at the last rib) was measured ultrasonically (Scanoprobe II, Scano, Ithaca, NY) on d 30, 70 and 109 of gestation, upon farrowing, and on d 5, 10, 15 and 20 of lactation. Live-weight was measured on the same days as backfat depth, and on the day of breeding and weaning. Litter size was standardized to at least nine piglets within 36 h of parturition by cross-fostering. Routine piglet procedures such as teeth clipping, tail docking, ear-notching, and iron injection were conducted 2 d post-partum. No creep feed was available to the piglets during lactation. Litters were weighed on d 0, 3, 5, 10, 15, and 20 of lactation, and at weaning. Milk production was assessed by

Table 3-1 Percentage composition of the lactating and gestating sow diets (as-fed)

Ingredient	Lactation Protein Loss[a] High	Lactation Protein Loss[a] Moderate	Lactation Protein Loss[a] Low	Gestation[b] diet
Wheat	30.0	30.0	27.0	-
Barley	-	-	-	45.8
Hulless Barley (Condor)	42.3	42.4	39.1	40.0
Soybean Meal (46 CP %)	7.0	10.0	20.0	4.4
Canola Meal	-	-	-	4.3
Fishmeal (Herring)	-	3.0	4.5	-
Sugar	10.0	5.0	-	-
Tallow	5.0	5.0	5.0	1.0
Iodized salt	0.6	0.6	0.6	1.5
Dicalcium phosphate	1.9	1.7	1.4	1.4
Limestone	1.1	1.2	1.2	0.6
Vit/Min Supplement [c]	1.0	1.0	1.0	1.0
Lysine HCl	0.07	0.07	-	-
Valine	0.01	0.08	0.16	-
Calculated analysis				
Energy, MJ ME/kg	14.10	14.10	14.21	12.53
Chemical analysis				
Crude Protein, %	11.85	15.06	19.77	11.79
Lysine, %	0.58	0.75	1.06	0.53
Valine, %	0.64	0.87	1.19	0.69

[a] Lactation diet formulated to 0.91% Ca, 0.75% P, and to induce sows to lose a low, moderate and high degree of body protein in lactation.

[b] Gestation diet formulated to 0.93% Ca, 0.70% P.

[c] The vitamin/mineral supplement supplied the following per kg of complete feed: 10,000 IU vitamin A, 1,000 IU vitamin D, 80 IU vitamin E, 2 mg vitamin K, 30 µg vitamin B_{12}, 12 mg riboflavin, 40 mg niacin, 25 mg D-pantothenic acid, 1,000 mg choline, 250 µg biotin, 1,600 µg folic acid, 5 mg ethoxyquin, 150 mg iron, 12 mg manganese, 120 mg zinc, 20 mg copper, 200 µg iodine and 300 µg selenium.

litter weight change in lactation. Milk samples (10 to 20 mL) were obtained twice from sows, after an i.m. injection of 10 I.U. of oxytocin on d 10, and on d 20 of lactation or 3 d prior to weaning, whichever came first. Milk samples were immediately stored at -20°C and later analyzed for protein, fat and lactose content. On d 107 of gestation, and on d 1, 5, 10, 15, and 20 of lactation, and at weaning, a single 10 mL blood sample was collected from the ear-vein of the sow prior to feeding in the morning. Feed was removed from the sows at 1800 the previous evening to ensure that sows had been fasted for at least 16 h prior to blood sampling. Blood was collected into a heparinized tube, spun at 1,500 x g for 15 minutes, and the plasma poured off and stored at -20°C for later insulin and IGF-1 analysis. Within 2 to 3 h of weaning sows were slaughtered. The liver was weighed after removal of the gall bladder, and approximately 200 g of hepatic tissue was collected from the same lobe, and stored at -20°C for later DM and N analysis. The reproductive tract was removed and the uterus trimmed of the mesenteric tissue, sectioned immediately below the cervix, and weighed. Both ovaries were collected and washed twice in sterile saline containing kanamycin (0.1 mg/mL; Sigma, St Louis, MO).

The number of follicles > 2 mm diameter on both ovaries was recorded from each sow as an index of the size of the pre-ovulatory follicle pool. The external diameter of the follicle was determined by recording a mean of two calliper measurements taken at 90° to one another, and follicles were categorized as either small (2.5 to 3.5mm) or medium (4.0 to 6.0mm) diameter. Follicular fluid was aspirated individually from the largest eight follicles on each ovary, using a 250 µl Hamilton syringe and collected. The syringe weight before and after aspiration was recorded, and the difference between these two weights was calculated as the follicular fluid weight. Follicular fluid volume was calculated assuming a density of 1 g/mL. Individual follicular fluid samples were diluted to 10% with tissue culture media (TCM 199 containing Earle's salts, L-glutamine and no sodium bicarbonate; GibcoBRL/Life Technologies, Grand Island, NY) and stored at -30°C in 1.5 mL micro-centrifuge tubes. An equal volume of diluted follicular fluid from the 12 largest follicles from each sow was pooled and filtered using a 0.2 µm filter (Millipore, Millipore SA, France) under a sterile hood. The dilute, sterile, filtered follicular fluid was stored at -20°C until required for in vitro culture of generic oocytes. The remaining follicular fluid samples were used for E_2 and IGF-1 analysis.

3.2.2 Analyses

i) Liver, feed and milk analyses. Liver samples were freeze-dried to constant weight, the dry weight was recorded, and the tissue was pulverized into powder. Sub-samples were used for DM and N analysis. Feed samples were ground in a Wiley mill through a 0.8mm screen, mixed well, and stored at 4°C until DM, N, and amino acid analysis. Feed and liver N was analyzed using the FP-428 Nitrogen Determinator, System: 601-700-900 (LECO Corporation, St. Joseph, MI). The amino acid composition of the feed was determined by HPLC (Sedgewick et al., 1991), except that methionine, cysteine, tryptophan, and proline were not determined. The concentrations of milk fat, protein and lactose were determined by infrared analysis using a MilkoScan Analyzer (Foss Electrics, Denmark) at the Alberta Central Milk Testing Laboratory (Edmonton, AB).

ii) Calculated nitrogen, lysine, and energy balance, milk production, and sow protein body mass. Energy, nitrogen, and lysine balance were calculated in lactation based on the recorded values of sow energy, lysine, and digestible nitrogen intake, minus the calculated requirements for sow maintenance and milk production. The sow's maintenance requirements were assumed to be 106 kcal ME/kg body-weight$^{0.75}$ (444 kJ ME/ kg body-weight $^{0.75}$) (NRC 1998), 0.38 g digestible N/ kg body-weight$^{0.75}$ (Mullan et al., 1993), and 0.039 g digestible Lys/kg body-weight$^{0.75}$ (Fuller et al., 1989). The digestibility of dietary lysine was assumed to be 86% (Mullan et al., 1989). The ME requirement for milk production (Energy$_{milk}$) was calculated from the equation of Noblet and Etienne (1989), modified by NRC (1998), and the dietary efficiency of ME use for milk production was taken as 72% (Noblet and Etienne, 1987). The digestible N requirement for milk production (N$_{Milk}$) was calculated from the equations of Noblet and Etienne (1989). An efficiency of use of dietary N for milk production of 70% was used (Noblet and Etienne, 1989). The N digestibility of the diets were assumed to be respectively 0.86, 0.88, and 0.90 for sows fed to achieve a low, moderate and high protein loss (Chapter 2). The total lysine requirement for milk production (Lysine$_{milk}$) was calculated from the equation of Pettigrew (1993).

$$\text{Energy}_{milk}\ (\text{kJ ME/d}) = [((4.92 \times \text{litter gain (g/d)}) - (90 \times \text{No. pigs}))/0.72] \times 4.184$$

$$\text{N}_{Milk}\ (\text{g/d}) = [(0.0257 \times \text{litter gain (g/d)}) + (0.42 \times \text{No. pigs})]\ /0.70$$

$$\text{Lysine}_{Milk}\ (\text{g/d}) = 26 \times \text{litter gain (kg/d)}$$

$$\text{Body}_{protein}\ (\text{kg}) = -20.4 + (0.21 \times \text{live-weight}) + (1.5 \times \text{backfat [P2]})$$

$$\text{Body}_{fat} = (\text{kg}) = -2.3 + (0.19 \times \text{live-weight}) - (0.22 \times \text{backfat [P2]})$$

It is difficult to directly determine body composition changes in domestic animals in real time. Sow whole-body protein (Body$_{protein}$) and fat (Body$_{fat}$) masses were thus estimated using the equations of Whittemore and Yang (1989), and lean tissue was assumed to be composed of 23% protein. Milk production was calculated using the equation of J. R.

Pluske (personal communication), based on the same genotype of first-parity sow in our herd (3.88 g milk/g litter gain).

iii) Plasma insulin, and plasma and follicular fluid IGF-1 analyses. Plasma insulin concentrations were analyzed by the double-antibody RIA described by Cosgrove et al. (1992). The sensitivity of the two assays was 0.019 ng/tube and the mean intra- and inter-assay CV were 5.6% and 11.9%. Individual follicular fluid IGF-1 concentrations, from each sow, were evaluated from diluted follicular fluid (10% in TCM 199) from the eight highest follicular fluid volumes. Plasma and follicular fluid IGF-1 concentrations were determined after acid-ethanol extraction, as described by Cosgrove et al. (1992). Based on an estimate of cold recovery of IGF-1 added to the standard plasma pool, the extraction efficiency was 100%. The double-antibody RIA of Glimm et al. (1990) was used with modifications described by Cosgrove et al. (1992). Assay sensitivity was defined as 92% of the total binding and the intra-assay CV was 11.2%.

iv) Follicular fluid E_2 analysis. The diluted follicular fluid (10% in TCM 199) from the eight highest follicular fluid volumes from each sow were further individually diluted 1:50 with PBS gel assay buffer ($NaH_2PO4 \cdot H_2O$, 2.77 mM; NaH_2PO4, 7.22 mM; NaCl, 139.7 mM; NaN_3 , 15.38 mM; and 0.1% [wt/vol] gelatin; Sigma). Diluted follicular fluid (final dilution 1:500) from the four highest and the next four highest follicular fluid volumes were pooled and used to measure E_2 concentrations using a Coat-A-Count RIA kit (Diagnostic Product Company, Los Angeles, California) with a minor modification. The kit Ab1 was diluted 3-fold in ddH_2O to improve assay sensitivity, which was defined as 95% of total binding and was 0.03 ng/mL. The intra-assay CV between 35 and 82% bound for the two assays were 4.0 and 5.0% and the inter-assay CV was 6.9%.

v) In vitro maturation of generic oocytes. Oocyte-cumulus cell complexes were matured in a standard system in vitro as described by Ding and Foxcroft (1994), with minor modifications (Zak et al., 1997b; Yang et al., 2000a). The follicular fluid volumes from follicles at weaning were only 25 to 50% of the follicular fluid volumes at proestrus. Therefore we only cultured one or two dishes (20 to 40 oocytes)/animal rather than up to six dishes (between 90 to 180 oocytes)/animal, as in the other experiments (Zak et al., 1997b; Yang et al., 2000a). Duplicate batches of 20 standardized oocytes were matured in vitro, in 1 mL of sterile diluted follicular fluid (10% in TCM 199) from each experimental sow. Culture was carried out under an atmosphere of 5% CO_2 in air, at 39°C for 46 ± 1 h. The degree of oocyte nuclear maturation and cumulus cell expansion was recorded as a percentage of the oocytes present. To determine the ability of diluted follicular fluid, from sows fed divergent protein and lysine intakes, to advance oocyte maturation, the usual additions of 100 µg/mL glutamine, 70 µg/mL L-ascorbic acid, and 35 µg/mL insulin were not included in the culture medium. These additions could mask any treatment effects due to changes in follicular fluid growth factors. The degree of cumulus cell expansion was evaluated and classified into four categories, from no or limited cumulus cells expansion to the highest degree of expansion, and used as an indirect index of oocyte cytoplasmic maturation (Sirard et al., 1988; Vanderhyden and Armstrong, 1989). Oocyte nuclear maturation was classified according to Hunter and Polge (1966) as either germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I (M I), or metaphase II (M II).

vi) Statistical analyses. All computations were performed using the GLM procedures of SAS (1990). Changes in sow live-weight and backfat depth were assessed over time (T) in gestation using repeated measures ANOVA. The model Y = FL + Rep was used, where Y = the measured response; Rep = the number of replicates in the experiment (n = 3); and FL = the feed level offered in gestation (n = 3). The effects of lactation treatment on changes in sow live-weight, backfat depth, feed intake, calculated N and energy balance, plasma hormone concentrations, and litter, milk and ovarian measures were assessed over time using repeated measures ANOVA. The model Y = FL + PL + Rep + FL x PL was used, where PL = the degree of body protein lost in lactation (n = 3). Litter and piglet growth rate measures included pig birth weight as a covariate in the model. In the event of a significant T x PL interaction, the differences among time within each treatment were computed using pre-defined orthogonal contrasts. Data for the ovarian variables, uterine weight, age at breeding, live-weight and backfat depth at farrowing and change over lactation, and litter and liver variables were analyzed by ANOVA using the latter model. If significant treatment differences were detected ($P < .05$), these were computed using Fisher's protected least significant difference test. If FL or the FL x T interaction was not significant ($P > .35$) it was removed from the model. Individual follicular IGF-1 content and concentration were analyzed for the largest eight follicular fluid volumes for each sow using the model Y = PL + Size + PL x Size + Sow(PL), where Size = follicle diameter (categories: 1 = $\leq$ 3mm; 2 = 3.25 to 4.5mm; and 3 = > 4.5mm). Follicular fluid E_2 content and concentration were analyzed for the largest four, and next largest four follicular fluid volumes for each sow using the model Y = PL + Rep + Sow(PL). Variation among the experimental units (sow within lactation treatment) was used as the estimate of experimental error, and for significance testing for follicular fluid E_2 and IGF-1 concentrations and contents.

3.3 RESULTS

Of the 36 bred gilts allocated to treatments, five gilts were not pregnant and six were taken off test due to illness, lameness or diarrhoea, in the litters. Thus 8 sows were evaluated on the low, 7 on the moderate, and 10 on the high protein loss treatments. The feeding regimens in gestation produced animals at parturition that had a uniform backfat depth (15.7 ± 1.1mm) and calculated fat mass (44 ± 1.9 kg), live-weight (196 ± 3.4 kg) and calculated protein mass (31.5 ± 0.7 kg). To account for the slight variation in live-weight and protein mass, animals from the three gestation feeding levels were randomly allocated to the three lactational treatments. Gestation feeding level was included as a dependent variable in the statistical model to test for differences in the variables measured during lactation. Variation in parturition protein mass had no effect ($P > 0.30$) on any of the variables measured in lactation; therefore, results for the gestation feeding treatment are not presented here. The litter size born (10.1 ± 1.2) and born alive (9.5 ± 1.3), piglet birth weight, and the number of pigs (~9 pigs) suckling sows throughout lactation were similar among treatments (Table 3-2).

There were no treatment differences in feed intake or energy intake in lactation overall (Table 3-3), or at any time throughout its course (Figure 3-1). Protein intakes on the three treatments were 878, 647 and 491 g/d, and lysine intakes varied correspondingly (Table 3-3 and Figure 3-1). The dietary treatments elicited a progressively larger ($P < 0.001$) degree of live-weight loss in lactation (Table 3-3 and Figure 3-2). However, backfat losses were minimal in lactation (-1.3 ± 0.29mm) and did not differ among treatments.

Table 3-2 Litter variables in first-parity sows that lost a high, moderate, or low amount of body protein during lactation

	Protein Loss in Lactation			
	Low	**Moderate**	**High**	**P**
Litter Size:				
d 0	9.4 ± 0.37[a]	9.5 ± 0.38	9.3 ± 0.36	0.902
d wean	9.1 ± 0.24	9.0 ± 0.25	9.0 ± 0.23	0.942
Piglet weight				
Birth, kg	1.65 ± 0.16	1.37 ± 0.16	1.63 ± 0.15	0.399
Wean, kg	6.04 ± 0.28	5.42 ± 0.29	5.90 ± 0.27	0.300
Piglet growth rate (GR), g/d				
d 0 to 20 (Period 1)	251 ± 8.9	244 ± 9.7	261 ± 8.6	0.452
d 15 to 20 (Period 2)	312 ± 12.7	305 ± 13.3	307.0 ± 11.8	0.940
d 20 to wean (Period 3)	287 ± 17.3	259 ± 18.2	228 ± 16.0	0.081
% Δ in GR, Period 1 to Period 3[b]	13 ± 5.7^{y}	7 ± 6.2^{xy}	-11 ± 5.4^{x}	0.019
% Δ in GR, Period 2 to Period 3[c]	-7.4 ± 5.3	-13.2 ± 5.7	-25.9 ± 4.9	0.060

[a] Least-square means ± standard error of the mean.

[b] Change in piglet growth rate between the first 20 d of lactation and the end of lactation (d 20 to wean) as a percentage of the piglet growth rate over the first 20 d.

[c] Change in piglet growth rate change between peak lactation (d 15 to 20) and the end of lactation (d 20 to wean), as a percentage of peak lactation piglet growth rate.

[xy] Within a row, means without a common superscript letters differ by the significance level in that row.

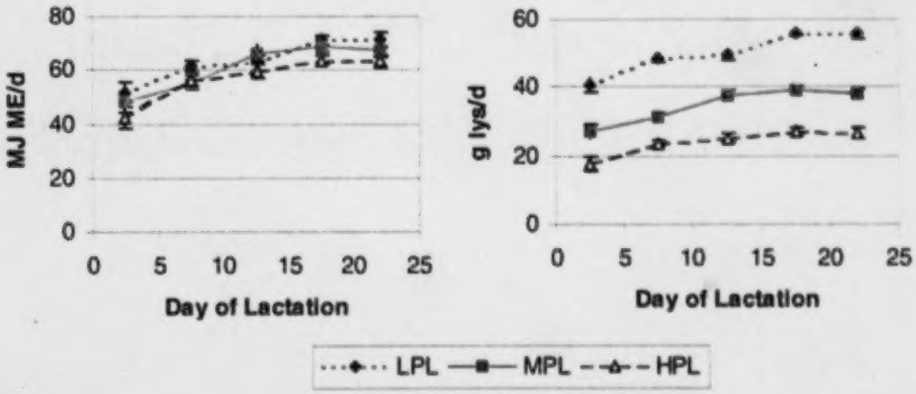

Figure 3-1 Metabolizable energy (MJ ME/d) and total lysine (g/d) intake throughout lactation in first-parity sows that lost a low (LPL), moderate (MPL), or high (HPL) amount of protein during lactation Both variables increased ($P < 0.001$) as lactation progressed, and lysine intake progressively decreased ($P < 0.001$) with increasing protein loss in lactation.

Table 3-3 Sow and nutrient variables in first-parity sows that lost a high, moderate, or low amount of body protein during lactation

	Protein Loss in Lactation			
	Low	Moderate	High	P
Feed Intake:				
kg/d	4.49 ± 0.14 ^{a}	4.33 ± 0.14	4.03 ± 0.13	0.103
MJ ME/d	63.7 ± 1.93	61.0 ± 2.01	56.9 ± 1.91	0.089
g CP/d	878 ± 19 ^{z}	647 ± 19 ^{y}	491 ± 18 ^{x}	0.0001
g Lys/d	50.2 ± 1.06 ^{z}	34.6 ± 1.10 ^{y}	24.2 ± 1.04 ^{x}	0.0001
Weight, kg:				
Farrow	195 ± 3.6	197 ± 3.8	200 ± 3.4	0.516
Loss in Lactation	12.9 ± 2.3 ^{x}	16.9 ± 2.4 ^{x}	28.4 ± 2.1 ^{y}	0.0003
Backfat, mm:				
Farrow	15.4 ± 1.18	15.0 ± 1.24	16.3 ± 1.10	0.712
Loss in Lactation	0.89 ± 0.32	1.45 ± 0.34	1.57 ± 0.30	0.340
Liver				
Weight, kg	2.83 ± .104 ^{y}	2.72 ± .111 ^{y}	2.31 ± .101 ^{x}	0.009
DM, g	778 ± 27.3 ^{y}	741 ± 29.0 ^{y}	630 ± 26.5 ^{x}	0.006
Protein, g ^{b}	588 ± 15.9 ^{z}	533 ± 16.9 ^{y}	428 ± 15.4 ^{x}	0.0001
Uterine weight, g	280 ± 16.9 ^{y}	231 ± 16.5 ^{xy}	216 ± 14.1 ^{x}	0.027

[a] Least-square means ± standard error of the mean.
[b] Liver protein calculated as 6.25 x N.
[xyz] Within a row, means without a common superscript letters differ by the significance level in that row.

Therefore, the difference in live-weight loss among treatments was most likely attributable to protein loss, and was estimated to represent 7, 9 and 16% of the sow's body protein mass at parturition (Table 3-4). At weaning, liver weight, DM, and protein mass ($P < 0.01$), and uterine weight ($P < 0.05$) also reflected the progressive amount of body protein lost in lactation, and were highest in sows that lost the lowest amount of protein (Table 3-3).

Piglet growth rate rose progressively over the first 20 d of lactation, peaked between d 15 and 20 and declined thereafter. There was no treatment difference in piglet growth rate over the first 20 d of lactation, or any interval thereof. Piglet growth rate diverged by treatment after d 20 of lactation, such that larger amounts of protein loss were associated with reduced piglet growth (Table 3-2). Sows that lost most protein showed the largest ($P < 0.02$) decline in piglet growth. The change in piglet growth rate at the end of lactation (d 20 to weaning) was respectively +13%, +7% and -11% of the average growth rate

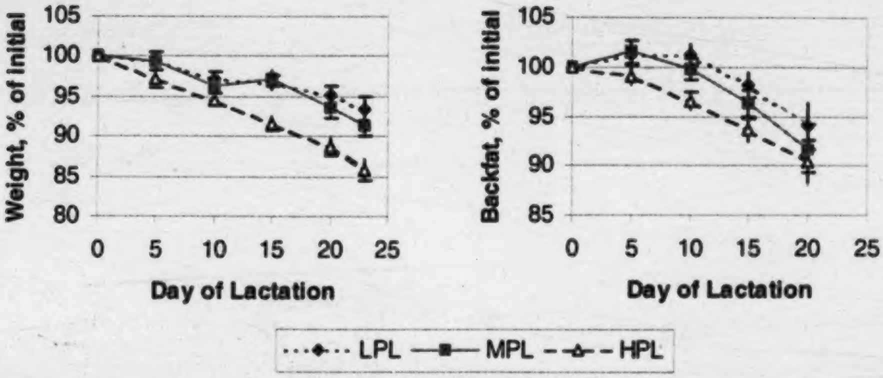

Figure 3-2 Changes in weight and backfat depth in first-parity sows that lost a high (HPL), moderate (MPL) or low (LPL) amount of protein during lactation. Live-weight and backfat depth changes were plotted as a percentage of the initial (parturition) value. Sow live-weight and backfat declined ($P < 0.0001$) as lactation progressed, and larger ($P < 0.0001$) decreases in live-weight were observed in sow that lost more protein in lactation.

during the first 20 d of lactation ($P < 0.02$) for the low, moderate and high protein loss sows. The change in piglet growth rate at the end of lactation (d 20 to weaning) was respectively -8, -13, and -26% ($P < 0.06$) of peak piglet growth. Furthermore, calculated milk production at the end of lactation was lowest ($P < 0.08$) in sows that lost the most body protein and was respectively 10.1, 9.0, and 8.0 kg/d in sows that lost a low, moderate and high amount of protein.

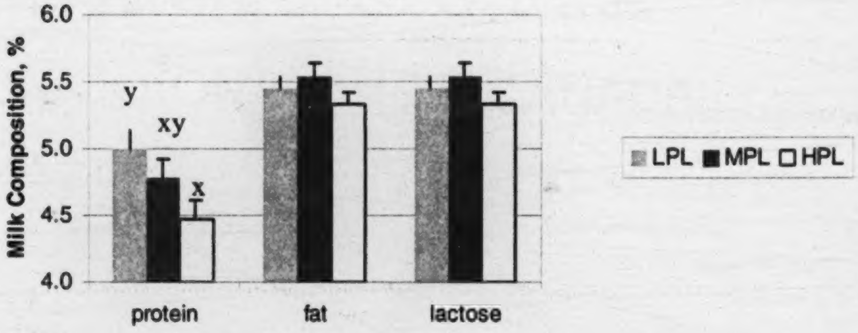

Figure 3-3 Percentage milk protein, fat and lactose composition on d 20 of lactation in first-parity sows that lost a high (HPL), moderate (MPL) or low (LPL) amount of protein during lactation.
^{xy} Values with different superscript letters within each parameter differ ($P < 0.05$).

Milk protein concentration at the end of lactation (d 20) also reflected the amount of body protein mobilized, and was lowest ($P < 0.05$) in sows that had mobilized the most protein in lactation (Figure 3-3). No difference was observed in milk lactose ($5.44 \pm 0.10\%$) and fat concentration among treatments at this time, or at any time in lactation. However, milk fat concentration declined ($P < 0.001$) between d 10 and 20 of lactation (7.81 ± 0.19 vs 6.80 ± 0.18).

The calculated N, lysine, and energy balances reflected the sow's dietary intake and the fact that piglet growth rate was essentially identical among treatments for the first 20 d of lactation (Table 3-4). All three balances decreased from early- (d 5) to mid-lactation (d 10) and remained at this level until d 20. After d 20 the pattern of N, lysine and energy balance among treatments diverged (Table 3-4). In sows fed to lose a low and moderate amount of protein the three balances did not change between d 20 and the end of lactation. These sows appeared to lose body protein at a relatively constant rate from d 10 of lactation until weaning. However, in sows that lost the most protein, all three balances became more positive after d 20; this was significant ($P < 0.01$) for lysine and tended to be different for N ($P = 0.09$) and energy ($P = 0.06$) balance (Table 3-4). The increase in N, energy, and lysine balance in these sows was achieved through a reduction in milk production and milk protein content (see above), enabling these elements to be retained to

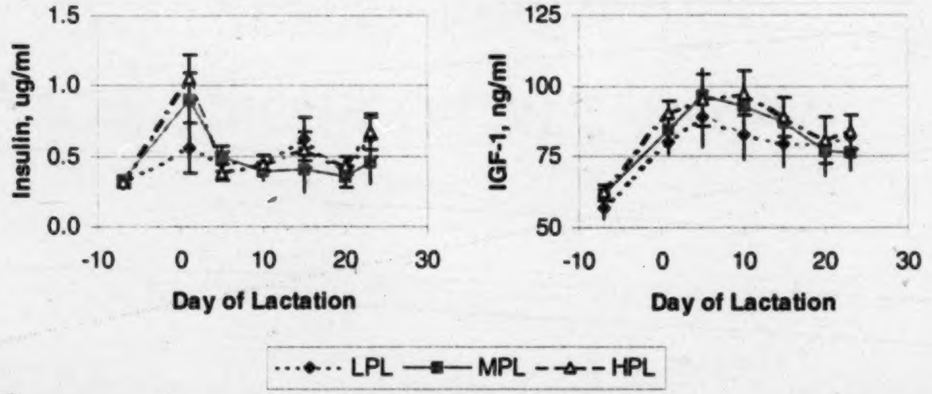

Figure 3-4 Plasma insulin and IGF-1 concentrations from late gestation (d 107) and throughout lactation in first-parity sows that lost a high (HPL), moderate (MPL) or low (LPL) amount of protein during lactation. Both plasma IGF-1 and insulin concentrations changed ($P < 0.001$) as lactation progressed.

Table 3-4 Calculated N, lysine and energy balances and protein body mass of first-parity sows that lost a high, moderate, or low amount of protein during lactation

	Protein Loss in Lactation			
	Low	**Moderate**	**High**	**P**
Body Protein Loss[a], % mass at parturition				
Cumulative loss to d 20	5.1 ± 1.42 ^{bx}	6.1 ± 1.48 ^{x}	12.3 ± 1.32 ^{y}	0.005
Cumulative loss to weaning	7.0 ± 1.56 ^{x}	9.2 ± 1.63 ^{x}	15.8 ± 1.45 ^{y}	0.003
Nitrogen Balance, g dig N/d				
d 0 to 20 (Period 1)	9.4 ± 3.2 ^{z}	-15.1 ± 3.4 ^{y}	-44.7 ± 3.0 ^{x}	0.0001
d 20 to wean (Period 2)[c]	11.8 ± 4.3 ^{y}	-15.4 ± 4.4 ^{x}	-25.3 ± 4.2 ^{x}	0.0002
Δ from Period 1 to Period 2[d]	2.4 ± 6.3	-0.3 ± 6.6	19.5 ± 6.2	0.093
Lysine Balance, g/d				
d 0 to 20 (Period 1)	-16.1 ± 1.8 ^{z}	-27.2 ± 1.9 ^{y}	-41.5 ± 1.7 ^{x}	0.0001
d 20 to wean (Period 2)[c]	-20.9 ± 2.8	-29.4 ± 2.9	-28.2 ± 2.8	0.134
Δ from Period 1 to Period 2[d]	-4.8 ± 3.0 ^{x}	-2.1 ± 3.1 ^{x}	13.0 ± 3.0 ^{y}	0.003
Energy Balance, MJ ME/d				
d 0 to 20 (Period 1)	-23.6 ± 4.0 ^{xy}	-20.3 ± 2.5 ^{y}	-29.2 ± 2.2 ^{x}	0.047
d 20 to wean (Period 2)	-25.1 ± 3.7	-22.1 ± 3.9	-16.0 ± 3.7	0.273
Δ from Period 1 to Period 2[d]	-4.0 ± 5.6	-2.2 ± 5.9	15.0 ± 5.5	0.056

[a] Lactational protein body mass predicted from the equations of Whittemore and Yang (1989).

[b] Least-square means ± standard error of the mean.

[c] Calculated N and lysine balance adjusted for varying milk protein concentrations among treatments at d 20 of lactation.

[d] Change in N, energy and lysine balance between the first 20 d and the end of lactation (d 20 to wean).

[xy] Within a row, means without a common superscript letters differ by the significance level in that row

a greater degree in the maternal body and exported to a lesser degree into the milk. At the onset of the shift towards maternal retention, these sows were estimated to have lost ~12% of their initial (parturition) protein mass (Table 3-4). The other two treatments had only lost an estimated 5 and 6% of their initial protein mass by d 20. Neither pre-prandial plasma insulin nor IGF-1 concentrations differed among treatments, but both hormones differed with time (Figure 3-4ab). Both hormones increased sharply ($P < 0.001$) between late gestation and the start of lactation, and IGF-1 remained higher than pre-partum levels throughout lactation. Pre-prandial insulin concentrations declined from d 1 to 5 of lactation to levels above those observed pre-partum, and remained at this level until d 20 when concentrations increased at weaning (Figure 3-4).

3.3.1 Ovarian Function

Table 3-5 Ovarian variables at weaning in first-parity sows that lost a high, moderate, or low amount of body protein during lactation

	Protein Loss in Lactation			
	Low	Moderate	High	P
Follicular Fluid Volume, µL				
Largest 16 follicles[b]	$47 \pm 5.0^{a\ y}$	45 ± 4.1^{y}	24 ± 3.9^{x}	0.003
Largest 8 follicle[c]	68 ± 7.7^{y}	55 ± 7.2^{y}	32 ± 6.8^{x}	0.006
Follicular fluid hormone concentrations[c]:				
E_2, ng/mL	3.82 ± 0.69^{y}	1.80 ± 0.64^{x}	1.47 ± 0.60^{x}	0.045
E_2, pg/foll	153 ± 25.0^{y}	96 ± 23.1^{xy}	41 ± 21.6^{x}	0.012
IGF-1, ng/mL	92 ± 13.5	84 ± 23.8	73.3 ± 16.8	0.683
IGF-1, ng/foll	5.1 ± 0.65	4.6 ± 1.2	2.6 ± 0.89	0.104

[a] Least-square means ± standard error of the mean

[b] Average follicular fluid volume from the largest 16 follicles (largest 8 from each ovary) measured at weaning, not including polycystic ovaries.

[c] Ovarian parameters were evaluated from the 8 largest follicular fluid volumes. The number of animals evaluated on the different treatments were respectively 7, 7, and 6 for LPL, MPL, and HPL sows (see results section for details).

[xy] Within a row, means without a common superscript letter differ by the significance level in that row.

Almost all the ovarian variables measured were lowest ($P < 0.05$) in sows that were fed to lose the most protein in lactation. Differences in the measured ovarian variables between sows that lost about 7 and 9% of their estimated whole-body protein mass in lactation were small and generally not significant (Figure 3-5abc and Table 3-5). There was no difference in follicle number in any size category and in follicular fluid volume either in the largest 16 or largest 8 follicles between the two treatments. There was no difference between the two treatments in follicular IGF-1 content and concentration or follicle E_2 content, and no difference in the ability of follicular fluid from these sows to advance in vitro oocyte nuclear maturation. Follicular fluid E_2 concentrations were lower ($P < 0.05$) in sows that lost about 9% compared to 7% of their estimated whole-body protein mass in lactation (Table 3-5). Also, a larger proportion ($P = 0.06$) of oocytes cultured in follicular fluid from these sows showed moderate cumulus cell expansion (Figure 3-5d).

By contrast, there was sharp demarcation in ovarian measures between sows that lost the most (about 16%) whole-body protein and those that lost about 7 and 9% of their estimated whole-body protein mass. Sows that lost the most protein had less ($P < 0.05$) follicles of > 2mm and > 4mm diameter compared to the other two treatments (Figure 3-

5a). Also both the largest 16 and 8 follicles from these sows had about half the volume of follicular fluid compared to the other two treatments (Table 3-5). Only 6 of the 10 sows that lost the most body protein could be evaluated for many of the in vitro ovarian

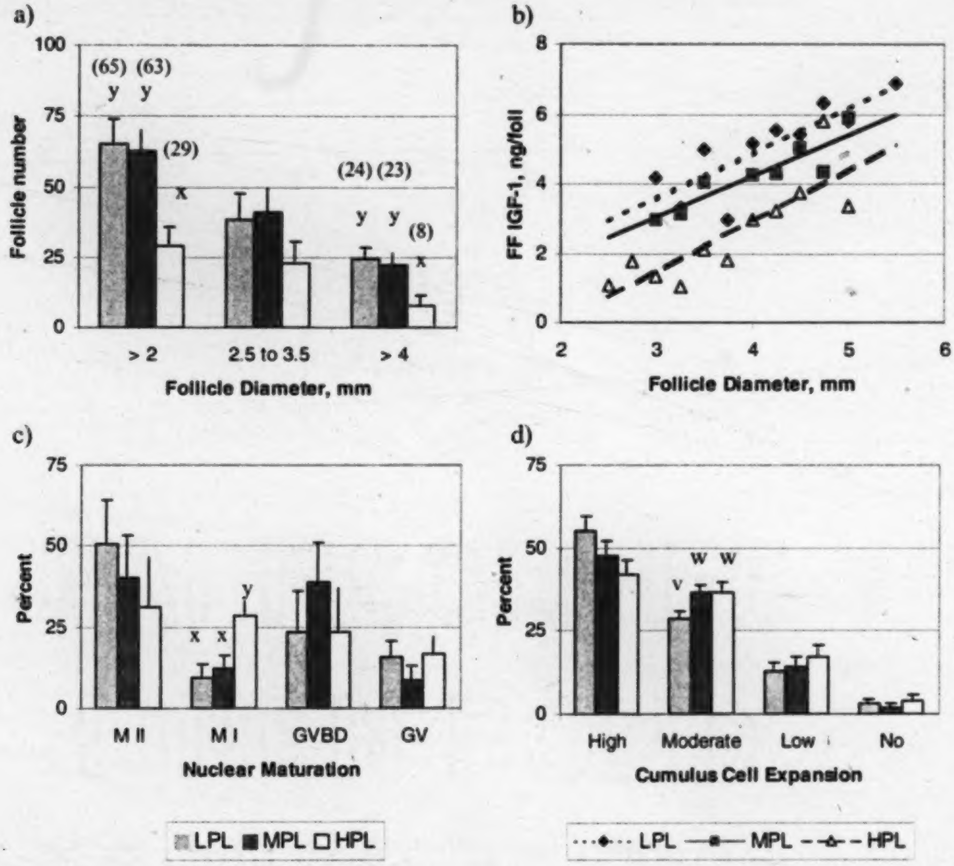

Figure 3-5 At weaning ovarian a) follicle number, b) follicular fluid IGF-1 content (ng/follicle), and the ability of follicular fluid to advance oocyte c) nuclear maturation, and d) cumulus cell expansion in first-parity sows that lost a high (HPL), moderate (MPL) or low (LPL) amount of protein during lactation. The number of animals evaluated for follicular IGF-1 content and the ability of follicular fluid to advance oocyte maturation were respectively 7, 7, and 6 for LPL, MPL, and HPL sows (see results section for details). Follicular IGF-1 content tended (P = 0.10) to be lower in HPL than LPL and MPL sows. Classification of cumulus cell expansion: No = no or minimal cumulus cell expansion and High = greatest cumulus cell expansion. Classification of nuclear maturation: M II = metaphase II; M I = metaphase I; GVBD = germinal vesicle breakdown; and GV = germinal vesicle.

^{vw} Values with different superscript letters within each parameter differ ($P < 0.06$)

^{xy} Values with different superscript letters within each parameter differ ($P < 0.05$)

measures. Four sows were excluded because insufficient follicular fluid could be collected from these sows to conduct determinations of hormone content and in vitromaturation; these sows had less than five medium-sized follicles (> 3.5mm diameter) on their ovaries at weaning. Nevertheless, follicular fluid from sows that lost the most protein was less able to advance in vitro oocyte nuclear maturation (Figure 3-5c), as indicated by a larger proportion ($P < 0.05$) of oocytes cultured in follicular fluid from these sows arresting at the M I stage of nuclear development. Follicular fluid from these sows had the poorest ability ($P < 0.06$) to support cumulus cell expansion (Figure 3-5d) and the lowest overall E_2 concentration and E_2 follicle content (Table 3-5).

3.4 DISCUSSION

The dietary design in this experiment allowed for the development of first-parity sows that were similar in live-weight and body composition at parturition. Although there was some variation in the animal's protein mass at parturition, this variation did not affect the interpretation of the main treatment effects observed during lactation. In lactation, sows were fed to lose three different amounts of their initial (at parturition) protein mass, by feeding iso-energetic diets that contained different levels of protein and lysine. Sows on the three dietary treatments respectively lost 12.9, 16.9 and 28.4 kg live-weight. The majority of this loss probably consisted of protein-containing tissue, since estimated losses of body fat were relatively small (-1.3 ± 0.29mm; -5.20 ± 0.76 kg). The calculated losses of body protein (lean) over lactation were of the order of 9, 13, and 22 kg lean tissue. An important feature of our study design was the absence of treatment differences in the level of fat loss, because it is difficult to design an experiment of this nature where no maternal fat loss occurs, due to the limitation of sow feed intake (Zak et al., 1998).

The animals in our study may be compared with those in several published reports. In some studies protein loss was accompanied by substantial fat loss (-6 to -8 mm backfat depth; -18 to -26 kg calculated fat). This makes it difficult to differentiate between the respective roles of fat and lean tissue loss on animal performance (King and Dunkin, 1985; Mullan and Williams, 1989, 1990). Other researchers employed an insufficient degree of protein restriction to impact on animal performance (Dourmad et al., 1998;

Touchette et al., 1998). While others used very severe protein restriction (6 to 17 g lys/d) such that milk production and litter growth rate were impaired throughout lactation (King et al., 1993; Jones and Stahly 1999a; Kusina et al., 1999; Yang et al., 2000b). Similar studies in the lactating rat also show a reduction in litter growth rate from early lactation (d 2) in animals fed a low protein (21 vs 9% CP) diet (Pine et al., 1994abc). The latter approach forces high rates of protein mobilization in early lactation, and suggests that there is an upper limit to the overall rate of protein mobilization in lactation. This can be deduced because the mobilization of protein from endogenous sources was insufficient to make up for the dietary protein deficit, and probably lead to the reduced milk production. Our approach revealed the proportion of protein loss associated with reduced lactational and reproductive performance in sows that were under similar nutritional conditions to those observed in production environments.

Measurement of body composition in lactating sows in real time is an expensive and difficult task to achieve. Furthermore, measurement of energy balance requires expensive equipment and is a daunting experimental task to conduct on lactating sows. For these reasons we used a number of indirect estimates of these variables, and equations to predict the protein and fat mass of the sows were also used (Whittemore and Yang, 1989). Although these equations are not derived from the same genotype of animal used in this experiment, they are derived from weaned sows that were still actively growing (parity 1 to 4) and which had similar backfat depths to the sows in this experiment. These equations therefore provide an estimate of the relative change in protein mass of the sow during lactation.

There are several limitations to the use of real N balances in estimating changes in protein mobilization (Manatt and Garcia, 1992). Similarly calculated N balances are also limited, for example if the dietary amino acid profile is non-ideal, as is the case for most lactation diets, then calculated N balances are overestimated. The calculated lysine balance is more representative of the sow's actual balance because 1) lysine is a limiting essential amino acid and invariably is not in excess in the lactation diet, and 2) unlike other essential amino acids (e.g branched chain amino acids) lysine is not retained to any great degree in the lactating mammary gland (Trottier et al., 1997; Bequette et al., 1997). While the absolute N balance may overestimate the calculated N balance, this does not affect interpretation of the results because the temporal pattern of calculated N and lysine balances were similar, and treatment differences were evident in both balances.

3.4.1 Litter performance

Milk production in the sow is a function of nutrient uptake by the mammary gland, and its biosynthetic capacity (Boyd et al., 1995). We controlled for the potential differences in mammary gland biosynthetic capacity by establishing similar litter sizes (~ 9 pigs) within 36 h of parturition, and by feeding gilts in gestation in a manner that would not be Sows fed the lower dietary protein intakes drew increasingly upon their protein reserves expected to affect fetal or mammary growth and development. The similarity in litter growth among treatments for the majority of lactation supports this claim. Therefore, a reduction in the supply of nutrients and energy to, and uptake by, the mammary gland is the main factor that could affect milk production in our experimental sows.

Sows fed the lower protein intake drew increasingly upon their protein reserves to meet the discrepancy between the demands of the lactating mammary gland and their dietary intake. At the end of lactation the decrease in litter growth in sows that had lost more than a calculated 12% of their protein mass was associated with an increase in N, energy and lysine balance. Because feed intake was not altered during this period, a reduction in the amount of body reserves mobilized and a parallel reduction in the amount of substrates delivered to the mammary gland would clearly have been necessary to cause a positive change in N, lysine and energy balance.

Protein metabolism undergoes important changes in lactation to allow preferential allocation of amino acids and other substrates to the mammary gland and to increase the efficiency of protein utilization. The ability of insulin to stimulate glucose and amino acid utilization by skeletal muscle in the ruminant is reduced in early lactation, at a time when nutrient demands are high (Debras et al., 1989; Tauveron et al., 1994). This adaptation allows more available amino acids and gluconeogenic substrates to be diverted towards the mammary gland. Such changes also probably occur in monogastric species, such as the pig, and could potentially be reversed at the time of critical protein loss. This could be determined in a series of new studies following key aspects of hormone-sensitive metabolism through the transition into more positive N balance.

Increased efficiency of protein utilization in non-mammary tissues (e.g. skin and liver) appears to be implemented in lactating animals that incur protein loss. The fractional rate of protein synthesis in skin (Baracos et al., 1991; Champredon et al., 1990) and skeletal muscle (Champredon et al., 1990) is lower in lactating compared to dry dairy goats, leading to a decrease in the absolute rate of protein synthesis in these tissues. The absolute rate of liver protein synthesis also appears to be lower in sows that lost the most body protein in lactation. This is suggested because the absolute rate of liver protein synthesis depends upon organ size (Baracos et al., 1991; Champredon et al., 1990; Pine et al. 1994bc). Also sows that lost the most body protein in lactation had smaller livers at weaning in this, and similar experiments (Brendemuhl et al., 1989; Sauber et al., 1998). In addition, sows in later lactation show a reduced urinary N output per unit of dietary protein absorbed (Noblet and Etienne, 1987; Chapter 2). This apparent reduction in hepatic amino acid catabolism could simply be related to a smaller hepatic mass or to a reduction in activity of catabolic pathways. It is presently unknown whether the aforementioned changes are reversed at the time of critical protein loss in order to conserve the remaining body protein stores. In Chapter 4 we describe indices of muscle protein metabolism relative to the cumulative protein loss in these animals.

3.4.2 Ovarian Function

If lactation length is approximately 20 d, ovarian follicles undergoing antral formation in early lactation are likely to constitute those recruited into the pre-ovulatory pool after weaning. These follicles could be affected by the nutritional state of the sow at any stage of lactation. This can be deduced because an antral follicle (≥ 400 μm diameter) may be recruited into the pre-ovulatory pool in the span of 19 to 21 d (Morbeck et al., 1992). Once follicles have been recruited into the pre-ovulatory pool they require a further 5 d to reach ovulatory size (≥ 8mm diameter).

The type of extreme dietary treatment imposed in this experiment results in reduced ovarian function post-weaning (Zak et al., 1997b; Yang et al 2000b). This is likely to be associated with decreased embryo survival (Zak et al., 1997a) and ultimately a lower subsequent litter size. Such a nutritional insult in lactation probably also reduces LH pulsatility in sows during lactation (Zak et al., 1997b; Yang et al 2000b). However, only a marginal (1 to 2 d) if any extension of the weaning to estrus interval may be observed in sows similar to those used in this experiment (Zak et al., 1997a, 1998; Mao et al., 1999; Yang et al., 2000b). Our choice of measured ovarian variables was intended to clarify key elements that could explain the changes in reproductive status observed under similar conditions of feeding and protein depletion. In the case of first-parity sows in our herd, the wean-to-estrus interval typically ranges between 3 and 6 d (Zak et al., 1997a, 1998; Mao et al., 1999). Therefore, the medium-sized follicles on the ovary at weaning (> 3mm diameter) are likely to be those from which the ovulatory follicles are selected.

In our experiment sows that lost the largest amount of protein had fewer medium-sized follicles on their ovaries at weaning and therefore would likely have had a smaller pre-ovulatory follicle pool. The literature supports the concept that ovarian variables are sensitive to nutrient intakes during lactation. Restrict feeding (~50% of ad libitum intake) for a 14 d (Miller, 1996) and 28 d (Quesnel et al., 1998) lactation was associated with fewer moderate sized (> 3 to 4mm diameter) ovarian follicles at weaning, and also lower LH pulsatility in lactation. Also sows fed low lysine (16 vs ≥ 36 g/d) levels in lactation had fewer large follicles (> 7mm diameter) at proestrus post-weaning and a lower LH pulsatility in lactation (Yang et al., 2000ab). The lower pre-ovulatory pool size may have implications for reproductive performance because these changes could underlie a lowered ovulation rate and this may ultimately contribute towards a reduced subsequent litter size.

The relative fertility of the sow can also be addressed by measuring indices of ovarian function at weaning. The degree to which follicular fluid is able to advance generic oocyte maturation reflects the ability of the oocytes within those follicles to undergo maturation in vitro (Zak et al., 1997b). This measure is therefore an indicator of the relative fertility of the animal. The lower ability of follicular fluid from sows that lost the most body protein to advance oocyte maturation could potentially be related to increased embryo mortality and eventually reduced subsequent litter size. In agreement with our results, follicular fluid taken at pro-estrus from first-parity sows fed 16 compared to 36 and 56 g lysine/d was less able to advance oocyte nuclear maturation in vitro, indicating that such sows were likely to have had a lower reproductive status (Yang et al., 2000a). In comparison to the present experiment, these sows were fed similar energy levels (30 to 34 MJ NE/d) and lost a similar degree of backfat (-1.4 to -2.2mm), but more live-weight

(-22 vs -19 and -15 kg) in lactation. Therefore, it is conceivable that these sows had also lost more than a critical level of protein in lactation. It is not apparent which signal(s) trigger the transition to conservation of maternal protein mass and impairment of ovarian function. Metabolic status among treatments did not differ, as assessed by circulating pre-prandial insulin and IGF-1 concentrations, so peripheral IGF-1 concentrations were not implicated. The potential role of other putative signals remains to be determined. The increase in pre-prandial insulin concentration in lactation no doubt reflects the higher energy intake of lactating compared to gestating sows. The similar insulin concentrations in lactation reflect the similar energy intakes and balances among treatments in lactation.

Ovarian follicular fluid contains many growth factors such as IGF-1, EGF, and TGF_β, as well as steroid hormones such as E_2, and these factors influence the oocyte's ability to be fertilized, develop into an embryo and survive (Osborn and Moor, 1983; Driancourt and Thuel, 1998). A lower follicular IGF-1 content may be implicated in the reduced follicular quality of the moderate-sized follicles in sows that lost more than a critical level of protein in our experiment and that of Quesnel et al. (1998). Certainly, elevated ovarian follicular fluid IGF-1 concentrations in the pig are associated with enhanced follicular development (Hammond et al., 1988) and lower follicular atresia (Matamoros et al., 1991).

3.4.3 Conclusion

Our data reveal that sows can sustain a loss of approximately 9 to 12% of their previously existing whole-body protein mass during lactation without any detriment to piglet growth, or the determined indices of ovarian function. Beyond this amount of protein loss a reduction of animal performance was observed. At the point when loss of about 12% of the sow's protein mass had been sustained, milk protein concentration and piglet growth rates started to decline. These animals also showed marked reductions in many indices of ovarian function at slaughter. A few slight reductions in ovarian variables were observed in the animals that had sustained about a 9% protein loss by the end of lactation. This suggests that the threshold for initiation of these alterations in animal performance was in the vicinity of 9 to 12% protein depletion. The decrease in piglet growth rate observed at a loss of about 12% of the sow's body protein was associated with a sharp increase in maternal N, lysine and energy balances and represents an apparent attempt by the sow to conserve maternal protein mass at the expense of piglet growth.

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CHAPTER FOUR

Changes in indices of muscle protein degradation and synthesis and free amino acid levels in first-parity sows fed to lose divergent levels of protein during lactation

4.1 INTRODUCTION

Lactation is a physiological state in the pig often associated with loss of protein from the maternal body (Mullan and Williams, 1989, 1990; Dourmad et al., 1998; Sauber et al., 1998). The capacity to mobilize body protein enables the sow to maintain lactational performance under conditions of poor nutrient supply (King et al., 1993b; Sauber et al., 1998). However, if large amounts of body protein are mobilized in lactation, litter growth rate and milk production decline (King et al., 1993ab; Kusina et al., 1999; Jones and Stahly, 1999a; Chapter 3). The subsequent reproductive performance of the sow also declines, as indicated by an extended wean-to-estrus interval, depressed LH pulsatility during lactation, and reduced ovarian function post-weaning (King, 1987; King and Martin, 1989; Jones and Stahly, 1999b; Yang et al., 2000ab; Chapter 3).

In gestation, growing animals are in positive N balance and accrete protein, but upon the transition from gestation to lactation N balance often becomes negative and protein is mobilized from body stores. Protein mobilization occurs when the rate of protein degradation exceeds that of synthesis, and may be achieved by increasing the rate of protein degradation, decreasing the rate of protein synthesis, or both. The relative contribution of muscle protein synthesis and degradation to protein mobilization over the course of lactation remains unclear. Protein mobilization is achieved in early lactation by increasing the rate of muscle protein degradation in the goat (Baracos et al., 1991; Tesseraud et al., 1993) and rat (Pine et al., 1994ab). If the degree of nutritional deficiency is severe enough in lactation, the fractional rate of muscle protein synthesis will also decline in the rat (Pine et al., 1994ab) and dairy goat (Champredon et al., 1990). In lactating sows the degree of myofibrillar protein breakdown increased relative to the degree of dietary lysine deficiency, and was higher throughout lactation (Jones and Stahly, 1999a; Yang et al., 2000b).

Sow feed intake, milk production and piglet growth rate change over the course of lactation in a characteristic manner, and the degree of maternal protein mobilization must be integrated with these factors. Over the first 10 to 14 d of lactation protein mobilization may increase as the sow's nutrient intake is less than her requirement for milk production. Thus, the sow's N balance is negative in mid-lactation in a manner directly related to dietary intake (King et al, 1993b; Chapter 2). In mid-lactation, nutrient intake becomes maximal, and litter growth rate and protein mobilization reaches a plateau. Protein may continue to be mobilized at a constant rate until weaning, but in sows which sustained a cumulative protein loss greater than 12% of that present at parturition, an increase in N and lysine balances was observed (Chapter 3). This change was achieved through a reduction in milk production and protein content, allowing protein to be retained to a greater degree in the maternal body and exported to a lesser degree into the milk. This shift towards maternal retention is potentially regulated at the level of muscle protein synthesis and degradation, to reduce protein mobilization.

Changes in muscle protein mobilization over the course of lactation are likely to depend on the degree to which the dietary protein supply supports the requirement for substrates for milk protein synthesis, and to the degree of cumulative protein loss. The larger the discrepancy between dietary supply and lactational demand, the greater the impetus for mobilization of muscle protein reserves. In later lactation, protein mobilization appears to be deliberately attenuated to preserve maternal protein mass, especially in animals that have mobilized more than a critical level of body protein. To fully understand these complex changes it is necessary to sequentially measure muscle protein synthesis and degradation prior to and over the course of lactation.

Classic techniques for determining muscle protein synthesis and degradation are technically and conceptually daunting in vivo, especially in large animals, and multiple measures within the same animal over time are precluded (Garlick et al., 1980; Lobley et al., 1980, 1992). To follow changes in muscle protein mobilization throughout lactation in the same animal, we developed a muscle biopsy procedure that can be conducted in the barn with minimal disruption to the sow and piglets. Using this approach, we obtained muscle samples from sows in late gestation, mid-lactation and late-lactation for the determination of indices of muscle protein synthesis and degradation and changes in the muscle free amino acid pool. Sows were fed three diets that were iso-energetic, but contained progressively lower levels of protein and lysine, to induce differential maternal whole-body protein loss of up to 16% over the course of lactation.

4.2 MATERIALS AND METHODS

These experiments were conducted in accordance with the Canadian Council of Animal Care Guidelines, and were approved locally by the Institutional Animal Policy and Welfare Committee.

4.2.1 Experimental Treatments and Measurements

i) Preliminary Experiment. We conducted a preliminary experiment to develop a muscle biopsy technique that could be conducted in the sow's farrowing stall in the barn, rather than in a surgery room. This minimizes stress of handling and changed environment, and minimizes the time that the suckling litter is absent from the sow. Sow feed intake, litter growth rate, and the general health of the sow were monitored to evaluate the success of this procedure. Four gilts were fed in gestation in a similar manner to gilts in the main experiment (Chapter 3). Gilt live-weight (196 ± 9.9 kg), backfat depth (17.1 ± 3.3mm), and age (350 ± 19.4 d) at farrowing were also similar to animals in the main experiment. In lactation, gilts were offered feed, for 1 h thrice daily, on an increasing scale, starting from 4 kg/d on d 1 and increasing 1 kg/d weekly. On average this resulted in gilts being fed daily in lactation 4.99 kg of feed, 67 MJ ME, 679 g CP, and 43 g total lysine. A muscle sample was collected from the long head of the triceps brachii muscle, from alternate sides of the animal, in late gestation (d 110 to 113), mid-lactation (d 14), and within 2 to 4 h after weaning. The samples were immediately trimmed of connective and adipose tissue, frozen in liquid nitrogen, and stored at -70°C for later RNA, DNA, and protein analysis. The evening prior to surgery feed was removed from sows at 1800 h to ensure sows were fasted for 16 h.

ii) Muscle Biopsy. The surgeries were carried out in the sow's individual farrowing pens, under general anaesthetic. Sows were anaesthetized with Pentothal (0.17 mL 5% sodium thiopental; Sanofi Animal Health, Victoriaville, Quebec) administered via an ear vein while the animal was restrained with a nose snare. Anaesthesia was maintained with a closed-circuit system of halothane (2%), oxygen (2.5 to 3.5 mL/min), and nitrous oxide (0.5 to 1.0 L/min), with dosage rates depending on the sow's body weight. Piglets were placed in a separate pen for the duration of the surgery and recovery period (40 to 60 min), and the surgery itself took 10 min. Pigs were placed back on the sow on recovery, and suckling commenced immediately. During surgery sows were placed in dorsal recumbency with the foreleg to be treated extended to expose the shoulder. The lateral surface of the shoulder and forelimb was shaved and scrubbed with Betadine solution (Ayerst, St. Laurent, Quebec). A skin incision, approximately 2 cm in length, was cut midway along the line between the deltoid tubercle of the humerus and the olecranon.

Needle biopsies were first attempted (Bergstrom, 1962; Evans et al., 1982), but these resulted in lower tissue yields (170 to 220 mg) than would be sufficient for the planned analyses. All subsequent muscle samples were collected using a muscle dissection technique that yielded 2 to 4 g of muscle tissue, as previously described (Chapter 2), with minor modifications. A skin incision of 5 to 6 cm was made, and muscle was excised from the long head of the *triceps brachii*. The triceps brachii is of mixed-fibre type in the pig (Uhrin and Liptaj, 1992; McAllister et al., 1997), and it is therefore likely to be representative of the majority of skeletal muscle in the body (Ariano et al., 1973; Maltin et al., 1989; Delp and Duan, 1996).

iii) Main Experiment. The experimental design, housing, and management of gilts during gestation and lactation are described in Chapter 3. Briefly, 36 pregnant gilts (Camborough, PIC) were fed 2.5 kg/d of a conventional barley-soybean diet (12.5 MJ ME/kg, 12.9 % CP, and 0.56 % lysine) once daily until d 29 of gestation. To achieve a similar live-weight (196 ± 3.4 kg) and backfat depth (15.7 ± 1.1mm) at parturition gilts were fed one of three feed levels (2.8, 2.5 or 2.3 kg/d) based on live-weight at breeding from d 30 of gestation. Upon farrowing, sows were randomly and equally allocated to be fed divergent levels of dietary protein (878, 647, and 491 ± 18 g/d) and total lysine (50, 35, and 24 ± 1.0 g/d), but a similar energy level (61 ± 2.0 MJ ME/d). Body fat and protein losses in lactation were calculated from the equations of Whittemore and Yang (1989). Dietary treatments induced sows to lose divergent (approximately -7, -9, and -16%) levels of body protein, as a percentage of their protein mass at parturition. Losses of backfat (1.3 ± 0.29mm) during lactation were small and not different among treatments. The largest (16%) protein loss was associated with a reduction in ovarian function at weaning. Furthermore, piglet growth rate declined in these sows after a cumulative loss of about 12% of their protein mass at parturition.

A muscle sample was collected from each sow by biopsy (see above) in late gestation (d 107 of gestation) and mid-lactation (d 9 to 12). A final muscle sample was collected from each animal on d 23 of lactation, at slaughter. At this time point piglets were removed from the sow and the sows were then slaughtered within 2 to 3 h for collection of tissue samples. The muscle samples were immediately frozen in liquid nitrogen and stored at -70°C until they were analyzed for RNA, DNA, protein, free amino acids, and mRNA by Northern hybridization analysis.

The RNA:DNA ratio can be used in muscle as an index of protein synthesis, because ribosomal RNA is the cell's protein synthetic machinery and accounts for the majority (> 80%) of the total RNA in the cell (Darnell et al., 1986). By dividing the total RNA in a tissue by an index of cell number in the given tissue (DNA) (Powell and Aberle, 1975), changes in the protein synthetic capacity/muscle cell unit can be made. The RNA:DNA ratio is highly correlated with changes in the rate of muscle protein synthesis in starvation and after feeding a protein-free diet for several days in the rat (Millward et al., 1973, 1974; Goodman and Ruderman, 1980).

The bulk of proteolysis in normal muscle, and degradation of the contractile proteins, involves an ATP-dependent system involving the co-factor ubiquitin and the proteasome complex (Attaix et al., 1998). This is thought to be the primary mechanism by which breakdown of muscle protein increases in association with diverse forms of atrophy. Increases in muscle mRNA encoding proteins of this pathway are associated with, and have been used as an index of, increased rates of protein degradation in a variety of states where muscle protein is mobilized (Attaix et al., 1998). These proteins include ubiquitin (Wing and Goldberg, 1993; Baracos et al., 1995; Medina et al., 1995), 14-kDa ubiquitin-conjugating enzyme (14-kDa E_2; Temparis et al., 1994; Wing and Banville, 1994; Voisin et al., 1996), as well as several subunits of the 20S proteasome (Temparis et al., 1994; Baracos et al., 1995; Medina et al., 1995; Voisin et al., 1996).

4.2.2 Analyses

Before analysis, individual muscle samples were pulverized in a mortar and pestle in liquid nitrogen and stored at -70°C until required for analysis.

i) Muscle free amino acids, RNA, DNA and protein analysis. About 25 mg of powdered muscle was homogenized in 1 mL of 3% (w/v) trichloroacetic acid (Sigma Diagnostics, St. Louis, MO), and then centrifuged at 2,800 x g. The supernatant fraction, containing tissue free amino acids, was analyzed by HPLC using orthopthaldialdehyde derivatization and ethanolamine as the internal standard (Sedgewick et al., 1991). Muscle RNA, DNA and protein were quantified using a modification of the Schmidt-Thannhauser procedure which allows separation of protein, RNA and DNA from the same sample (Munro and Fleck, 1966). In brief, 250 mg of frozen powdered muscle tissue was incubated for 10 min on ice with 4 mL of 2% (w/v) perchloric acid (PCA) to precipitate out the proteins and nucleotides. The precipitate was mixed and centrifuged for 15 min at 2,800 g and 4°C, and washed with 4 mL of 2% PCA at 4°C as above. The pellet was re-suspended in 4 mL of 0.3N NaOH, and incubated at 37°C for 1 h. The alkaline digest was cooled on ice, and 100 μL removed and diluted with distilled de-ionized H_2O to make a 0.1N NaOH alkaline digest. This was stored at -20°C until used for protein determination. Two milliliters of ice-cold 12% PCA was added to the remaining alkaline digest and incubated on ice for 10 min to completely precipitate out the proteins and DNA. The sample was centrifuged for 10 min at 2,800 g at 4°C, and the supernatant containing the muscle RNA was collected. The pellet was washed in 4 mL of 2% PCA, and the sample was centrifuged, as above, and the washings and pellet collected separately. The acid-soluble supernatant plus washings were diluted with distilled de-ionized H_2O to make a 1% PCA solution, and muscle RNA was quantified from this spectrophotometrically using the formula of Ashford and Pain (1986): RNA (ug/mL) = $(32.9\ A_{260} - 6.11\ A_{232})$ x dilution factor. The remaining acid-insoluble precipitate was incubation in 10% PCA at 70°C for 1 h, according to Forsberg et al. (1991), to extract the muscle DNA. The DNA was quantified using the diphenylamine method (Burton, 1956; Giles and Myers, 1965). Protein was quantified using the micro-titre plate method of the BCA assay following the manufacturer's protocol (Pierce, Rockford, IL).

ii) Muscle RNA isolation and Northern Hybridization Analysis. The riboprobe for ubiquitin was kindly provided by Dr. J. Walker (University of Wisconsin-Madison). The cDNA probe for the 14-kDa E_2 was kindly provided by Dr. S. S. Wing (McGill University), and that of the proteasome subunit C9 was kindly provided Dr. A. Kumatori (Kumatori et al., 1990). The cDNA probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH or housekeeping gene) was generated by RT PCR, kindly provided by G. Murdoch (University of Alberta), using primers designed according to Yelich et al. (1997). The 14-kDa E_2 sequence was inserted at the SmaI site in the multicloning region in pTZ18R (Pharmacia Biotech Inc, Baie d' Urfé, Quebec) plasmid backbone, and the UBQ4 gene clone was inserted at the Kpn site in a pGEM-4 plasmid (Promega, Madison, WI). The plasmids were transfected into XLI-Blue MR supercompetent cells (Stratagene, La Jolla, CA), and the transformed bacteria were grown in LB broth at 37°C overnight. The plasmids were isolated from the bacteria using the PlasmidPure Miniprep kit following the manufacturer's protocol (Sigma, St Louis, MO). The cDNA fragments were isolated by digestion with restriction endonucleases (GibcoBRL/Life Technologies, Gaithersberg, MD) and purified from the agarose gel using the GeneClean kit following the manufacturer's protocol (Bio 101 Inc.).

Total RNA was extracted from 200 to 300 mg of muscle tissue using TRIzol™ (Gibco), according to the manufacturer's protocol (Chomczynski, 1993). Assessment of the purity and quantitation of RNA were determined by absorbance at 260 and 280 nm. Ethidium bromide (1.7 µg) was added to each muscle sample in the sample buffer, and samples were made up to the same volume with the addition of sterile distilled deionized H_2O. Samples were loaded separately onto 1% agarose-formaldehyde gels (Sambrook et al., 1989), which were run at 100 V for 5 h. The RNA was checked visually for intactness of the 28S and 18S rRNA and equivalent amounts of RNA loading. The amount of total RNA to load for each probe was tested by running concentration curves of increasing levels of total RNA, from muscle from lactating sows, and hybridizing blots from these gels with the respective probes. Muscle mRNA expression of both 14-kDa E_2 transcripts increased linearly ($r^2 > 0.95$) from 5 to 20 µg of total RNA. and thereafter reached a plateau (Figure 4-1), therefore 15 µg of total RNA were loaded for this hybridization. Muscle C9 mRNA expression increased linearly ($r^2 = 0.998$) from 15 to 42 µg of total

RNA (Figure 4-1), therefore 30 µg of total RNA was loaded onto a 1.2% agarose-formaldehyde gel for this hybridization. Muscle mRNA expression of both ubiquitin transcripts plateaued between 5 and 10 µg of total RNA, therefore 3 µg of total RNA was loaded for this hybridization.

Muscle RNA to be hybridized with the cDNA probes (C9, GAPDH, and 14-kDa E_2) were transferred onto nitrocellulose membrane (Nitropure, Micro Separations Inc., Westborough, MA), and RNA to be hybridized with the riboprobe for ubiquitin was transferred onto nylon membrane (Zeta-Probe, Bio Rad Labs, Hercules CA). Transfer was accomplished by capillary action for 16 h, and the RNA was fixed onto the membranes by baking in a vacuum oven for 2 h at 80°C. The cDNA probes were labeled to high specific activity using the Random-Primer-DNA-labeling kit, according to the manufacturer's protocol (Gibco). Membranes were pre-hybridized for 1 to 2 h in the

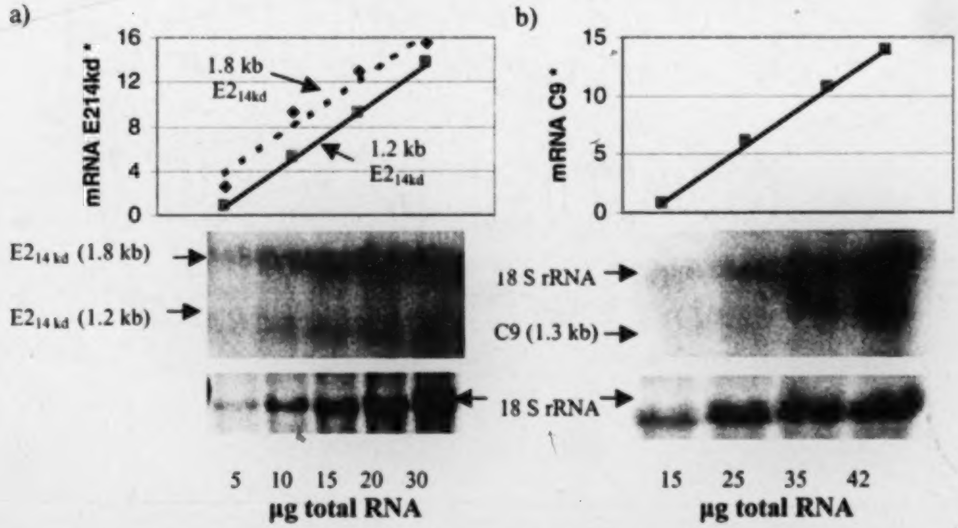

Figure 4-1 Lactating sow muscle mRNA expression concentration curves and Northern-blot analysis of total RNA (indicated by membrane 18 S rRNA ethidium bromide stain) from muscle hybridized against either a) both 14-kDa ubiquitin-conjugating enzyme ($E2_{14kd}$) transcripts (1.8 and 1.2 kb) and b) the proteasome subunit C9. The mRNA transcripts were expressed in arbitary densitometric units (*). The mRNA expression increased linearly with increasing levels of total RNA loaded for both $E2_{14kd}$ transcripts to 20 µg (1.8 kb:$Y = 0.845x - 0.465$, $r^2 = 0.954$; 1.2 kb:$Y = 0.861x - 3.59$, $r^2 = 0.999$), and for C9 ($Y = 0.486x - 6.410$; $r^2 = 0.998$).

presence of 0.5% sodium dodecyl sulphate (SDS), 5 x Denhardts solution, and 6 x SSPE at 65°C for 14-kDa E_2 and GAPDH, and 55°C for C9 (Sambrook et al., 1989). They were then hybridized overnight under the same conditions as the pre-hybridization, but with the addition of the ^{32}P-labelled cDNA probe and 100 μg/mL of denatured tRNA. The membranes were initially washed with 2 x SSPE and 0.1% SDS for 10 min at room temperature, and then in 0.1% SSC and 0.1% SDS at the respective hybridization temperatures. The riboprobe for ubiquitin was labeled using the in vitro Riboprobe System T7 labeling kit (Promega). Membranes were pre-hybridized at 50°C for 1 to 2 h in the presence of 60% deionized formamide, 1 x SSPE, 0.5% blotto, 10% dextran sulphate, 1% SDS, and 0.5 mg/mL denatured salmon sperm DNA. The hybridization tubes were initially soaked in 0.1N NaOH for 10 min to destroy any RNases present. Membranes were then hybridized overnight, under the same conditions as the pre-hybridization but with the addition of the ^{32}P-labelled riboprobe (2×10^6 dpm/ml). Membranes were rinsed in 2 x SSC, and washed for 30 min in 2 X SSC and 0.1% SDS at room temperature, and then in 0.2 x SSC and 1% SDS at 70°C. All blots were exposed to X-ray film (Kodak) for varying amounts of time, dependent on the probe, and evaluated quantitatively using a Model GS-670 Imaging Densitometer (Bio Rad).

iii) Statistical Analyses. All computations were performed using the GLM procedures of SAS (1990). Responses over time (late gestation, mid-lactation, and weaning) were analyzed by repeated measures ANOVA, with sow considered as the experimental unit. In the preliminary experiment only time was included in the model. In the main experiment treatment effects on the various muscle parameters were assessed using the model: $Y = Rep + L + G + G \times L$. Where Y = the measured response; Rep = number of experimental replicates (n = 3); L = lactation treatment (n = 3); and G = the gestation feed level (n = 3). Membrane was included in the model for the mRNA expression data. In the event of a significant ($P < 0.05$) time (T) or T x L interaction, differences were computed using pre-defined orthogonal contrasts. To test for differences among lactation treatments in the T x L interaction, the absolute or percentage difference between the two time periods was analyzed by ANOVA. If lactation treatment was significant in the repeated measures ANOVA for any parameter, differences among lactation treatments in late gestation in that parameter were tested for. If no difference among treatments was observed in late gestation, then the values within treatment over the three time periods were averaged, and an ANOVA was performed on the mean value. If significant treatment differences were detected ($P < 0.05$), then these differences were computed using Fisher's protected least significant difference test. Because the gestational feeding regimen had no effect ($P > 0.35$) on any parameters measured in lactation, results for the gestation treatment are not presented here.

4.3 RESULTS

4.3.1 Preliminary Experiment

The muscle biopsy was successfully conducted on all animals in no more than 10 minutes with minimal disruption to sow and piglets. Piglets were separated from the sow for one suckling bout only (40 to 60 min). The relatively high litter growth rates (2.36 ± 0.23 kg/d) achieved by these sows in lactation support the suggestion that multiple biopsies can be conducted with minimal impact on sows and piglets. Animal temperatures were not elevated post-biopsy, and feed intake was only slightly reduced. On the day of surgery, for the biopsy during gestation, feed intake was reduced by about half in two of the four gilts, and did not change for the other two gilts. Feed intake resumed thereafter to pre-biopsy levels. On d 14 of lactation, feed intake on the day of the biopsy was about 70% of that over the prior 5-d period (5.35 ± 0.35 kg/d). Feed intake then increased to pre-biopsy levels or above within 1 to 3 d. Sows only lost a small amount of live-weight (5.0 ± 5.3 kg) and backfat (3.3 ± 1.2mm) in lactation and these changes are in the range that would be expected for animals in our herd that were not subjected to biopsy.

4.3.2 Main Experiment

i) Changes in muscle RNA, DNA and protein concentrations. The RNA:DNA ratio or capacity for muscle protein synthesis decreased by about 15% between late gestation and mid-lactation ($P < 0.05$) in all treatments. The ratio continued decreasing in sows that lost the highest degree of protein only, and was 68% of pre-partum levels at weaning in these sows (Figure 4-2a). The protein:DNA ratio or amount of protein/muscle cell unit decreased ($P < 0.001$) between late gestation and weaning in all treatments (Figure 4-2b). The size of this decline reflected ($P < 0.10$) the degree of protein loss in lactation induced by the dietary treatments, and resulted in protein:DNA ratios at weaning that were respectively 84, 77, and 69% of pre-partum levels in sows fed to lose a low, moderate and high degree of protein. Muscle RNA concentrations decreased ($P < 0.001$) by about 10 to 15% between late gestation and mid-lactation in all treatments. The concentrations then increased to pre-partum levels by weaning in sows that lost about 7 and 9% of body protein but remained low in sows that lost ~16% of body protein (Figure 4-2c). Muscle DNA concentrations increased ($P < 0.0001$) by about 5% between late

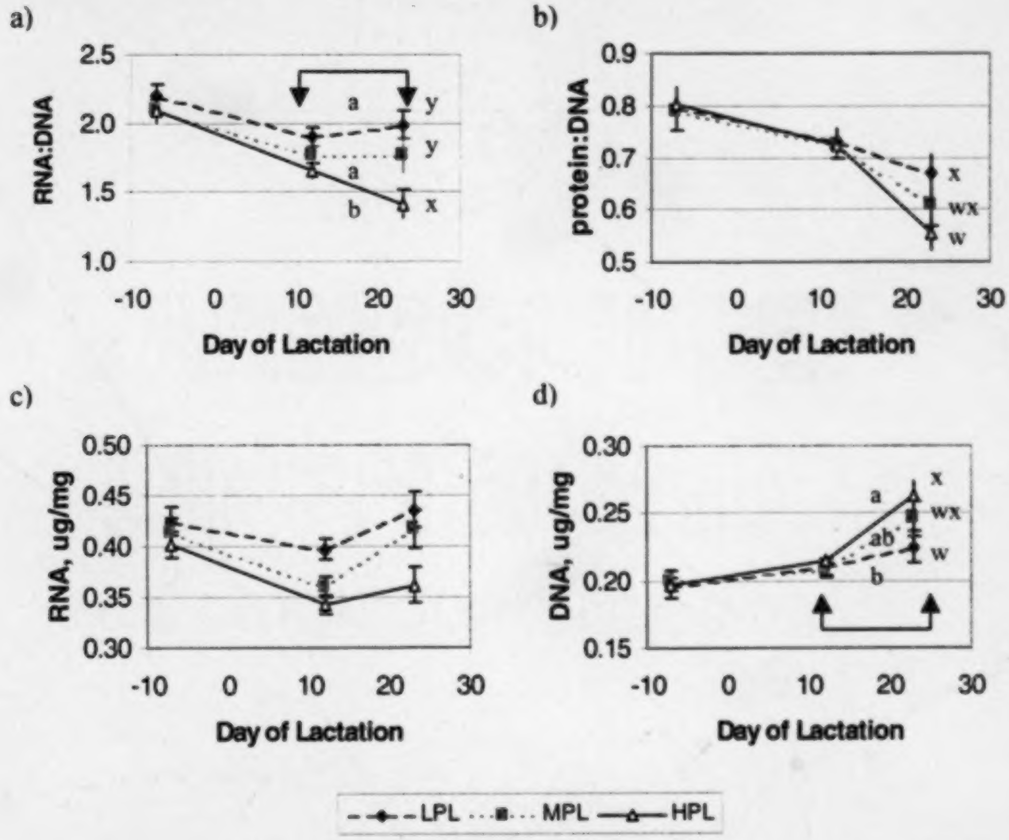

Figure 4-2 Changes in muscle a) RNA:DNA ratio, b) protein:DNA ratio, c) RNA concentration, and d) DNA concentration, from d 107 of gestation until weaning, in first-parity sows that lost a high (HPL), moderate (MPL) or low (LPL) amount of protein in lactation. Muscle RNA concentrations and RNA:DNA ratio differed ($P < 0.05$) with lactation treatment. All variables changed ($P < 0.001$) with time over lactation. Variables on d 23 without a common letter differ, xy ($P < 0.05$) and wx ($P < 0.10$).

[ab] The change in muscle variables between mid-lactation and weaning without a common letter differ ($P < 0.05$).

gestation and mid-lactation in all treatments, and then increased between mid-lactation and weaning in a manner that reflected the degree of protein lost in lactation (Figure 4-2d). The increase was largest ($P < 0.05$) in sows that lost the highest degree of protein resulting in the DNA concentration at weaning being 134% of pre-partum levels in these sows.

ii) *Changes in muscle C9, 14-kDa E_2, ubiquitin, and GAPDH mRNA expression.* When equal amounts of total RNA from each animal and time point was studied, marked changes in GAPDH mRNA levels were observed over the course of lactation and also by treatment (Figure 4-3). Expression of GAPDH fell by nearly 50% ($P < 0.001$) between late gestation and mid-lactation and remained low until weaning. Also, the overall GAPDH mRNA level was lowest ($P < 0.01$) in sows that lost the greatest degree of protein in lactation. The presence of significant changes in GAPDH mRNA precluded use of this factor as a "housekeeping" gene that could be used for normalization of results. Therefore, the measured genes mRNA expression was corrected for slight differences in loading using the relative fluorescence of the 18S rRNA ethidium bromide stain.

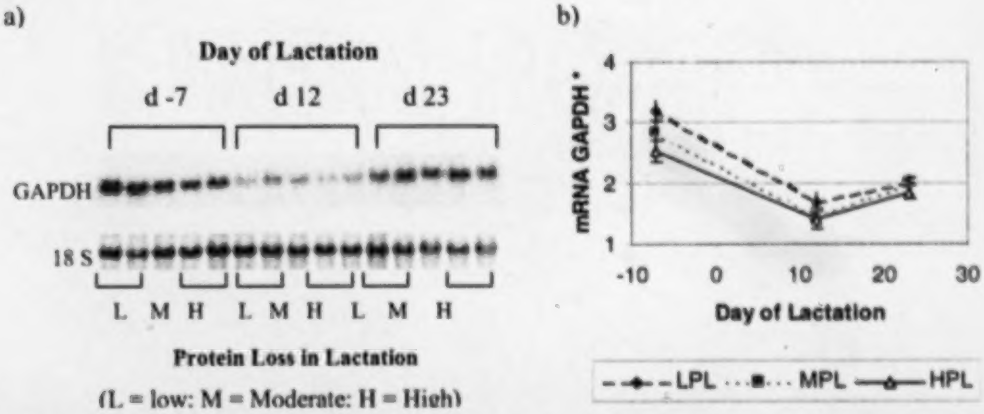

Figure 4-3 Muscle glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression during lactation, as a) a Northern-blot hybridized against GAPDH from porcine triceps muscle (15 μg) taken immediately pre-partum (d –7), at mid-lactation (d 12), and at weaning (d 23); and b) quantification of GAPDH mRNA, expressed in arbitrary densitometric units, from first-parity sows that lost either a high (HPL), moderate (MPL), and low (LPL) amount of protein during lactation. The level of total RNA loaded is indicated by the inverted 18S rRNA ethidium bromide staining. Muscle GAPDH mRNA expression decreased ($P < 0.001$) between late gestation and mid-lactation, and differed ($P < 0.05$) among lactation treatments.

As the levels of muscle mRNA did not differ among treatments in late gestation (pre-partum), gene expression data are presented as a percentage of pre-partum values. Muscle mRNA levels of several key elements of the ATP-ubiquitin-proteasome dependent proteolytic pathway in muscle increased as lactation progressed (Figure 4-4). Many of the changes in mRNA expression between late gestation and lactation reflected the degree of protein mobilization elicited by the dietary treatments in lactation, such that at weaning expression was highest in sows that lost the highest degree of protein (Table 4-1). The 1.2 kb transcript of the 14-kDa ubiquitin conjugating enzyme E_2 more than doubled between late gestation and mid-lactation in all treatments. Between mid-lactation and weaning the increase in expression was highest ($P < 0.05$) in sows with the highest degree of protein loss, and were respectively 0, 60 and 120% of mid-lactation mRNA expression values in sows that lost a low, moderate and high degree of protein (Figure 4-4a). The 1.8 kb transcript of the 14-kDa ubiquitin conjugating enzyme E_2 did not change between late gestation and mid-lactation in any treatment. Levels of this transcript almost doubled ($P < 0.05$) between mid-lactation and weaning in sows that lost the highest degree of protein, but did not change over this interval in the other two treatments (Figure 4-4b). Overall the 1.2 kb ubiquitin transcript was highest ($P < 0.05$) in sows with the highest degree of protein loss, and was respectively 0.40, 0.37 and 0.28 ± 0.030 arbitrary densitometric units in sows that lost a high, moderate, and low degree of protein (Figure 4-4c). The 2.5 kb ubiquitin transcript increased between 40 and 60% over lactation in all treatments, and although there were no significant treatment effects, the expression tended to be lowest in sows that mobilized the lowest degree of protein (Table 4-4d). Expression of the proteasome subunit C9 mRNA also increased ($P < 0.001$) between late gestation and weaning. This increase tended ($P = 0.09$) to be highest in sows that lost the highest degree of protein (Figure 4-4e), and was reflected in a higher ($P < 0.01$) C9 expression at weaning in these sows (Table 4-1).

iii) Changes in muscle free amino acid concentrations. The chromatographic procedure used allowed for resolution of 19 amino acids. The average concentration of individual muscle free amino acids in late gestation is presented in Table 4-2. Because muscle free amino acid concentrations did not differ among treatments in late gestation the amino acid data are presented as a percentage of pre-partum values. The muscle free amino acids were divided into two groups based on their pattern of change between late gestation and weaning. The first group consists mainly of essential amino acids (EAA; leucine, isoleucine, valine, phenylalanine, lysine, and threonine). Several essential amino

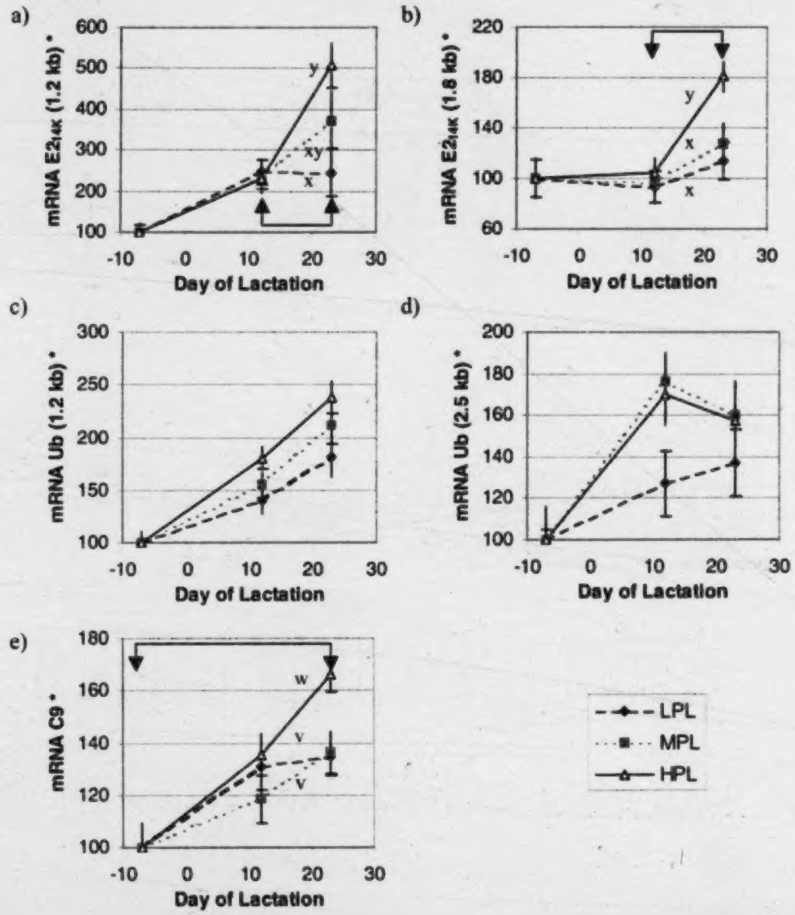

Figure 4-4 Muscle mRNA expression of a) the 1.2 kb, and b) 1.8 kb transcripts of ubiquitin-conjugating enzyme (14-kDa E2), and c) the 1.2 kb, and d) 2.5 kb transcripts of ubiquitin (Ub), and e) the 1.3 kb transcript of the proteasome subunit C9, from d 107 of gestation until weaning, in first-parity sows that lost a high (HPL), moderate (MPL), or low (LPL) amount of protein during lactation. The mRNA* expression was normalized to 18S rRNA, and expressed as a percentage of pre-partum values. All muscle variables increased ($P < 0.001$) over lactation. Overall ubiquitin 1.2 kb transcript mRNA expression differed among treatments ($P < 0.05$) and was highest in HPL, intermediate for MPL and lowest in LPL sows.

[xy] The increase in mRNA expression of both 14-kDa E2 transcripts, between mid-lactation and weaning, without a common letter differ ($P < 0.05$).

[vw] The increase in C9 mRNA expression, between late gestation and weaning, without a common letter tend to differ ($P = 0.06$).

Table 4-1 Muscle mRNA expression^{a} of the ubiquitin-ATP-dependent proteasome proteolytic pathway at weaning (d 23) in first-parity sows with divergent protein losses

	Protein loss in lactation			
	Low	**Moderate**	**High**	**P**
mRNA expression, d 23^{a}				
Ubiquitin, 2.5 kb	0.79 ± 0.06^{b}	0.88 ± 0.07	0.88 ± 0.06	0.554
Ubiquitin, 1.2 kb	0.40 ± 0.04^{x}	0.49 ± 0.04^{xy}	0.54 ± 0.03^{y}	0.048
14-kDa E2^{c}, 1.8 kb	0.83 ± 0.11^{x}	0.90 ± 0.11^{x}	1.40 ± 0.09^{y}	0.001
14-kDa E2^{c}, 1.2 kb	0.68 ± 0.16^{x}	0.90 ± 0.18^{x}	1.37 ± 0.14^{y}	0.011
C9, 1.3 kb^{d}	10.9 ± 0.44^{x}	10.3 ± 0.47^{x}	12.9 ± 0.41^{y}	0.002

[a] mRNA expressed in arbitary densitometric units and normalized to 18S rRNA.

[b] Least square mean ± standard error of the mean.

[c] Two transcripts (1.8 and 1.2 kb) of the 14 kDa ubiquitin-conjugating enzyme (14-kDa E2).

[d] The proteasome subunit C9.

[xy] Means without a common superscript letter differ by the significance level in that row.

The number of sows allocated to the three lactational treatments were: low protein loss (n = 8), moderate protein loss (n = 7), and high protein loss (n = 10).

acids, most notably methionine and histidine, were not included in the group because a value for these amino acids was not obtained for every sow at every time point. Arginine was not included in the group because its concentrations did not change over lactation. The second group consisted mainly of non-essential amino acids (NEAA; alanine, glycine, serine, aspartic acid, asparagine, and glutamic acid). Muscle free glutamine was excluded from this group because it behaved in a different manner to the other amino acids within the group.

Most muscle free amino acid (NEAA, EAA and glutamine) concentrations increased ($P < 0.001$) by about 30% between late gestation and mid-lactation (Figure 4-5a). A divergence in the pattern of free EAA and NEAA concentration then occurred between mid-lactation and weaning. At weaning, muscle free NEAA concentrations remained elevated above pre-partum levels. Their concentrations either did not change (alanine, glycine, and serine) or slightly declined (asparagine, aspartate, and glutamate) between mid-lactation and weaning. In contrast muscle free glutamine concentrations continued to increase sharply ($P < 0.001$) over this time period at more than twice the rate observed between late-gestation and mid-lactation. This resulted in free glutamine concentrations at weaning being more than double their pre-partum values (Figure 4-5a). Muscle free taurine and citrulline concentrations were similar in late gestation and mid-lactation and increased ($P < 0.05$) about 25% between mid-lactation and weaning.

Table 4-2 Triceps muscle free amino acid concentrations in gilts (n = 25) in late gestation (pre-partum)

Amino Acid	(μmol / mg wet tissue)	SD
Essential Amino Acids		
Arginine	2515	422
Histidine	53	11.2
Isoleucine	85	13.0
Leucine	108	18.8
Lysine	50.2	19.1
Methionine	21	8.7
Phenylalanine	55	10.1
Threonine	238	81.2
Valine	186	22.2
Non-Essential Amino Acids		
Alanine	161	34.0
Aspartic Acid	161	34.0
Asparagine	39	7.4
Glutamic Acid	898	203.9
Glutamine	694	108.8
Glycine	771	161.3
Ornithine	45	19.9
Serine	96	16.4
Taurine	1282	232.8

By contrast EAA concentrations declined ($P < 0.0001$) between mid-lactation and weaning to be slightly above (lysine and histidine), at the same level as (valine, isoleucine, and tryptophan) or below (leucine, phenylalanine, and threonine) pre-partum levels. Unlike the other EAA measured muscle free methionine and threonine concentrations did not change between late gestation and mid-lactation, and then declined 30% to weaning. This decline was significant ($P < 0.05$) for threonine but only numerical for methionine.

The increase in muscle free NEAA concentrations between late gestation and mid-lactation (Figure 4-5b), and in muscle free glutamine concentrations between late gestation and weaning (Figure 4-5c) reflected the degree of protein mobilized from the sow in lactation. Increases in these amino acids were highest ($P < 0.05$) in sows that lost the highest degree of protein. The NEAA concentrations in mid-lactation were respectively 122, 131, and 146% of pre-partum values, and the glutamine concentrations at weaning were respectively 205, 240 and 308% of pre-partum values in sows that lost a low, moderate and high degree of protein. The decrease in muscle free EAA concentrations between mid-lactation and weaning, such as isoleucine, was greatest ($P < 0.05$) in sows that had mobilized about 16% rather than about 7 and 9% of their estimated protein mass (Figure 4-5d). Similarly muscle free valine concentrations at weaning were lowest ($P < 0.05$) in sows that had mobilized about 16% rather than about 7 and 9% of their estimated body protein mass, and were respectively 79 ± 9% and 100% of their prepartum values.

4.4 DISCUSSION

N balance experiments have been the main approach used to study protein metabolism in the lactating pig (Noblet and Etienne, 1987; King et al., 1993; Dourmad et al., 1998; Chapter 2). The comparative slaughter technique was used to establishe that sows mobilize muscle protein in lactation (Whittemore and Yang, 1989; Mullan and Williams, 1990; Dourmad et al., 1998; Sauber at al., 1998). However, these techniques do not elucidate the physiological mechanisms causing maternal protein loss. As skeletal muscle is the main source of mobilizable protein in times of nutritional stress (Allison et al., 1963; Allison and Wannemacher, 1965; Swick and Benevenga, 1977), our experiment focused on characterizing changes in this tissue between late gestation and lactation in animals that lost varying amounts of body protein. Changes in muscle protein mobilization over the course of lactation are likely to depend upon the degree to which the dietary protein supply supports the requirement for substrates for milk protein synthesis, and also the amount of cumulative protein loss achieved by the animal. We therefore studied animals at key time intervals throughout the course of lactation, and used dietary treatments to induce progressively larger amounts of body protein loss. This mobilization of body protein reserves was estimated to be approximately 7, 9 and 16% of that present at parturition.

We developed a muscle biopsy technique that can be conducted in the barn with minimal disruption to the sow and piglets, and used this technique to collect muscle samples in late gestation and lactation; a final sample was collected at slaughter immediately after weaning. We measured indices of the rates of muscle protein synthesis and degradation and changes in the concentration of the muscle free amino acid pool. This study design enabled us to postulate how: 1) muscle protein metabolism alters between late gestation and lactation, 2) muscle protein metabolism changes once a sow starts to conserve maternal protein, and 3) loss of a fraction of a sow's protein mass impinges on her lactational performance and ovarian function.

4.4.1 Changes in muscle variables between late gestation and lactation

i) Total RNA and mRNA levels. We standardized our autoradiograms for uneven loading among samples using the relative abundance of 18S rRNA, rather than another

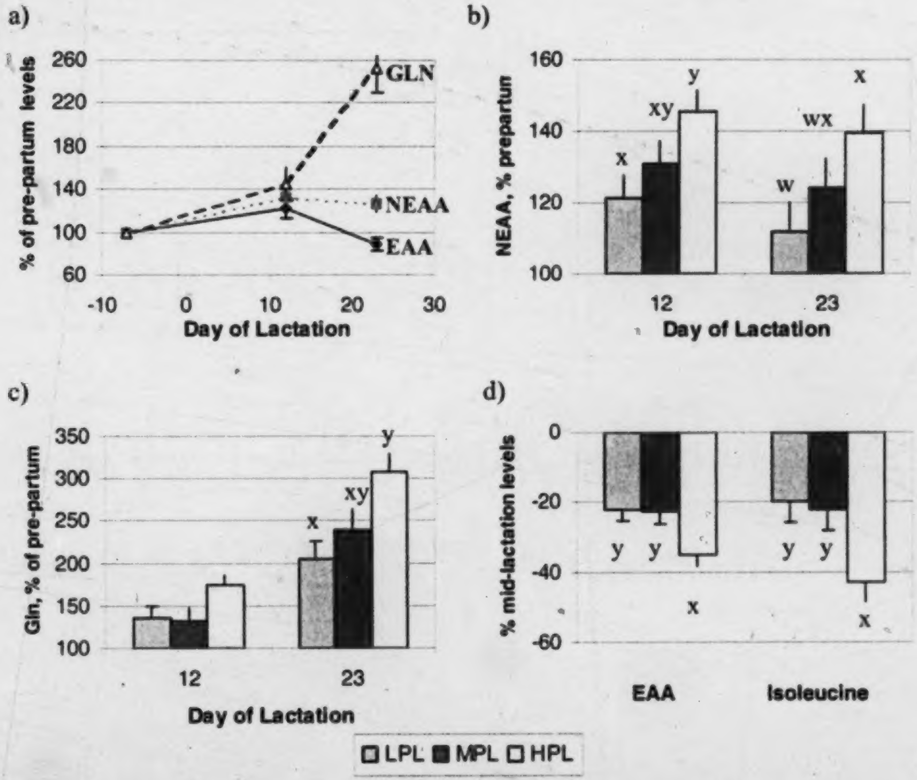

Figure 4-5 Changes in free muscle: a) non-essential (NEAA) and essential (EAA) amino acid and glutamine (Gln) concentrations from late gestation (pre-partum, d 107 of gestation) to late lactation (d 23), as a percentage of pre-partum levels; treatment differences in b) NEAA, and c) glutamine concentrations in mid- (d 12) and late-lactation, as a percentage of pre-partum levels, in first-parity sows that lost a high (HPL), moderate (MPL) or low (LPL) amount of protein; and d) EAA and isoleucine concentrations in late-lactation, expressed as a percentage of mid-lactation concentrations. NEAA include: ala, gly, ser, asp, asn, and glu. EAA include: leu, ile, val, phe, lys and thr. Percentage amino acid levels without a common letter differ, xy ($P < 0.05$), and wx ($P < 0.10$).

commonly used gene, GAPDH. A "housekeeping" gene in quantitative RNA analyses acts as an internal control and should be constitutively expressed and independent of experimental conditions. While this is true of GAPDH in muscle over a broad range of physiologic and pathologic conditions, we observed a dramatic decrease in GAPDH expression between gestation and lactation, in all treatments. This is not unexpected since alterations in GAPDH expression have been observed in vivo and in vitro, and are related to insulin levels (Nasrin et al., 1990) and hypoxia (Zhong and Simons, 1999). However, these changes precluded using GAPDH mRNA level as a basis for normalization of gene expression in our data. Because the polymerase that transcribes rRNA differs from that which transcribes mRNA, rRNA levels are less likely to vary under conditions that affect the mRNA expression.

We provided evidence that up-regulation of the ubiquitin-ATP-dependent proteasome proteolytic pathway occurs at the level of gene expression in muscle during lactation compared to late gestation. Increases in mRNA encoding ubiquitin, 14-kDa ubiquitin-conjugating enzyme E2, and a proteasome subunit, C9 were observed. The most marked increase was for the 14-kDa ubiquitin-conjugating enzyme E_2. Up-regulation of the main proteolytic pathway in muscle (Attaix et al., 1998), at the level of gene expression, suggests that the rate of muscle protein degradation was higher in lactating compared to gestating sows. This inference is supported by several studies in the lactating first-parity sow. Even in early lactation (d 1 and 4) the fractional rate of muscle protein degradation was at least 15% higher in sows fed low compared to high (< 36 vs > 55 g/d) lysine intakes (Jones and Stahly 1999a, Yang et al., 2000b). These differences were maintained until the end of lactation (d 15 and 20). Similarly in the rat and dairy goat, the main cause of increased protein mobilization in early-lactation is an increase in the rate of muscle protein degradation (Baracos et al., 1991; Tesseraud et al., 1993; Pine et al., 1994ab).

Changes in the rate of muscle protein synthesis help provide control and sensitivity to the degree of protein mobilized in lactation. For example, without changing the rate of muscle protein degradation protein mobilization can be increased by decreasing the rate of muscle protein synthesis. We provided evidence for a decrease in the capacity for protein synthesis in muscle in mid-lactation. By weaning, this variable had changed further in a manner related to the degree of protein mobilized. These changes in the capacity for protein synthesis may underlie a decrease in the rate of muscle protein synthesis. This inference is supported by data in the rat and dairy goat in which the rate of muscle protein synthesis was measured by either a flooding or tracer dose of [^{3}H] phenylalanine or L-[^{35}S] methionine. Compared to dry goats no reduction in the fractional rate of muscle protein synthesis was observed in goats in early-lactation that mobilized ~28 g protein/d (Baracos et al., 1991), but a 30% reduction was observed in goats that mobilized ~57 g protein/d (Champredon et al., 1990). Similarly, no reduction in the fractional rate of muscle protein synthesis was observed in rats that lost less than 5% of their body weight during lactation. But more than a 25% reduction in the fractional rate, and over a 45% reduction in the absolute rate of muscle protein synthesis was observed in rats that had lost more than 20% of their live-weight (Pine et al., 1994ab).

Decreasing the insulin sensitivity of non-mammary tissues, such as skeletal muscle, provides another mechanism by which the body can divert amino acids and other gluconeogenic substrates away from non-mammary tissues and towards the mammary gland. Insulin is less able to stimulate glucose utilization in skeletal muscle in goats in early-lactation compared to in non-lactating animals (Debras et al., 1989). The large reduction in muscle GAPDH mRNA in lactating compared to gestating sows suggests that the glycolytic pathway is down-regulated in muscle tissue in lactating sows, at least at the level of gene expression. Muscle of a mixed-fibre type is the main source of mobilizable protein in the rat during starvation and injury (Baille and Garlick, 1991; Fang et al., 1998) and in the pig during lactation (Brendemuhl et al., 1989). Glycolytic muscle fibres appear to be targeted for degradation in muscle undergoing protein mobilization (Henriksson, 1990), therefore the decrease in muscle GAPDH expression we observed could also reflect a reduction in the size of the glycolytic relative to the oxidative fibres in this muscle type. In related studies, the proportion of oxidative fibres in mixed-fibre muscle increased due to exercise or electro-stimulation. This was reflected in an increased muscle mitochondrial content (indicated by a higher citrate synthase activity), and a decreased activity of glycolytic enzymes such as GAPDH (Hood and Pette, 1989; McAllister et al., 1997).

ii) Changes in free muscle amino acids. The muscle free amino acid pool reflects the balance between the net rate of amino acid appearance from myofibrillar protein breakdown and the rate of amino acid export out of the tissue into the blood. Amino acids are exported out of the muscle free pool for use by the mammary gland for milk biosynthesis and other mammary functions in lactation. The composition of the amino acid mixture originating from myofibrillar protein in skeletal muscle is altered predominantly by conversion of the branched-chain amino acids (BCAA) valine, leucine and isoleucine to alanine and glutamine. Thus the amino acid mixture released from muscle is relatively enriched in alanine and glutamine, and relatively depleted in the BCAA (Lindsay, 1980; Newsholme and Leech, 1983). How well the amino acid mixture released from muscle matches that required by the mammary gland may be assessed by comparing the proportion of amino acids released from muscle with the proportion of amino acids taken up by the lactating mammary gland in the pig (Trottier et al., 1997). No data are available for the composition of the amino acid efflux from muscle in lactating pigs, however the amino acids entering blood from limb protein mobilization during the post-absorptive state in humans are available, and are similar across several species (Felig and Wahren, 1971; Felig et al., 1973). A comparison of mammary amino acid uptake and muscle amino acid release suggests that larger proportions of many NEAA (especially glutamine) and some EAA (e.g. lysine and histidine) are released from muscle than are taken up by the lactating mammary gland. This discrepancy is very large for some amino acids, such as glutamine, for which muscle release may be 5-fold higher than mammary uptake. In such a situation amino acids would be in excess in the whole-body free amino acid pool. This may explain why some amino acids rise in the muscle free pool over the course of lactation, and in a manner related to the protein mobilization rate. By contrast, smaller proportions of many EAA such as the BCAA, phenylalanine and threonine are released from muscle than are taken up by the lactating mammary gland. This may explain the reduction in their concentration in muscle because such free amino acids would be in deficit in the body.

The increase in muscle free glutamine concentrations in lactation was an interesting observation that is supported by other research. Wu and Knabe (1994) observed a similar increase in free glutamine concentrations in sow's milk over the course of lactation. Milk free glutamine accounted for respectively 8, 14, 16, 21, and 31% of the total free amino acid concentration in milk on d 3, 8, 15, 22 and 29 of lactation. Interestingly, the lactating black bear loses more muscle protein during hibernation (Tinker et al., 1998) and has 30% higher plasma glutamine concentrations (411 vs 299 nmol/mL) compared to the non-lactating hibernating bear (Wright et al., 1998).

Most muscle free amino acid concentrations increased 40 to 70% between late gestation and early lactation (week 3) in the dairy cow. However, in contrast to our results muscle free glutamine concentrations decreased 30% over the same time period. The low free muscle glutamine concentrations persisted until at least wk 15 of lactation (Meijer et al., 1995). This may be explained by the difference in physiology between ruminant and monogastric animals. In ruminants such as the dairy cow and goat, the supply of propionate from the rumen is insufficient to provide all the demands for glucose in early lactation (Bell et al., 2000). Therefore such animals up-regulate their capacity to convert alanine and other gluconeogenic amino acids, such as glutamine, to glucose (Overton et al., 1998). Thus the lactating ruminant has other important uses for glutamine, and this may explain the lower concentration of free glutamine in lactating ruminant muscle.

4.4.2 Changes in muscle metabolism upon loss of divergent degrees of body protein

A loss of more than 50% of skeletal muscle mass is considered fatal (Newsholme and Leech, 1983), and it would be expected that increasing cumulative loss of muscle protein would at some point trigger metabolic warning signals. We have observed a sharp reduction in protein mobilization in lactating sows upon loss of approximately 12% of their body protein mass. Maternal N balance became less negative, indicating a reduction in protein mobilization, while growth of the litter of piglets slowed. This degree of protein loss was also accompanied by a decline in ovarian function, and these physiologic changes to delay or prevent a further cycle of reproduction would also contribute to preservation of the remaining maternal protein mass. However, the positive shift in N balance seen in the whole animal was not associated with a reduction in the measured indices of muscle protein synthesis and degradation. Many of the genes in the ubiquitin-ATP-dependent proteasome proteolytic pathway in muscle were higher and the capacity for protein synthesis in muscle at weaning was lower in sows that lost the most protein.

The further up-regulation of the ubiquitin-ATP-dependent proteasome proteolytic pathway and the decrease in the capacity for protein synthesis in muscle of sows that had mobilized the most protein must be considered in light of the fact that as lactation progresses the muscle protein mass is rapidly shrinking. Therefore in order to release the same total amount of amino acids per unit of time muscle net catabolism rate must continuously increase. It may be estimated that the sow's muscle mass declined as lactation progresses to be at weaning respectively ~84, 80, and 64% of the muscle mass at parturition in sows fed to lose a low, moderate and high degree of protein. It may be that mobilization of muscle protein has a maximal rate, and that once this is attained the overall daily rate of amino acid delivery from muscle shrinks to the point that it is inadequate to sustain milk production. Remembering that the protein quality of this mixture is relatively poor (i.e. low in several essential amino acids required at high levels by the mammary gland), the supply of several essential amino acids may become first-limiting for mammary protein synthesis. In support of this hypothesis, induced deficiency of a single essential amino acid (histidine) in the lactating goat reduced milk protein output by about 20%, despite an approximately 33% increase in mammary blood flow (Bequette et al., 2000).

4.4.3 Conclusion

Up-regulation of the ubiquitin-ATP-dependent proteasome proteolytic pathway occurs at the level of gene expression in muscle in the lactating compared to gestation sow, suggesting that in lactation muscle protein mobilization is achieved by increasing the rate of muscle protein degradation. If the degree of protein mobilization is sufficiently high a decrease in the capacity for protein synthesis is also likely involved in the increased muscle protein mobilization. Changes in these processes reflect the degree of muscle protein mobilization. Once a sow has mobilized more than a certain proportion (~12%) of her protein mass in lactation litter growth, and by association milk production, declines. This may be due to a deficiency in the availability of several essential amino acids (e.g. BCAA, tryptophan, phenylalanine, and threonine) for uptake by the mammary gland. Unlike other non-mammary tissues, muscle tissue appears to continue to mobilize protein to a high degree throughout lactation, independent of the cumulative amount of body protein lost from the sow. The signal(s) that cause the reduced ovarian function, and probably the associated impairment of the hypothalamo-pituitary-ovarian axis in these sows has still to be elucidated. However, it is likely to be related to the degree of protein loss and the physiological changes that in general promote protein conservation in such sows. The contribution of changes in fat reserves to reproductive performance cannot be totally discounted in this experiment, but they are likely be minimized because lactational fat losses were similar among treatments.

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CHAPTER FIVE

Parturition body size and body protein loss during lactation influence performance during lactation and ovarian function at weaning in first-parity sows

5.1 INTRODUCTION

Excessive body protein loss from sows during lactation is associated with a reduction in reproductive performance (King, 1987; King and Martin, 1989; Jones and Stahly, 1999b), and in milk production and litter growth (Kusina et al., 1999b; Jones and Stahly, 1999a). First-parity sows are especially vulnerable to such reductions in performance because these animals have a relatively similar level of milk production to older sows but a smaller appetite (Aherne and Williams, 1992), and a smaller body protein mass.

In our recent study, gilts of a similar age, live-weight, calculated whole-body protein mass (31.5 ± 0.7 kg) and backfat depth, were fed three levels of dietary protein during lactation and mobilized an estimated 2.2, 2.9 and 4.9 kg of their whole-body protein reserve (Chapter 3). The most catabolic sows had a lower ovarian function at weaning, and were less able to sustain lactational performance. By the end of lactation, milk protein concentration and litter growth fell in these animals, and there was an overall shift towards protection of the maternal protein mass at the expense of piglet growth. The decline in animal performance was estimated to take place under conditions where protein from endogenous sources (primarily skeletal muscle) provided a large proportion of the protein required for milk protein synthesis and mammary function. In the least catabolic animals mobilized protein was estimated to account for less than 20% of the overall free amino acid supply for milk protein synthesis. But mobilized protein may have accounted for as much as 50% of the overall free amino acid supply for milk protein synthesis in the most catabolic animals.

The amino acid mixture released upon mobilization of skeletal muscle (hind-limb) is rich in alanine and glutamine (collectively represents about 60% of total released amino acids) and is relatively depleted in the branched-chain amino acids (BCAA, collectively represent about 9% of total released amino acids) (Felig, 1975). However, the BCAA's comprise approximately 20% of the amino acid mixture taken up by the mammary gland of the lactating sow, but glutamine, glutamate and alanine only comprise about 28% of this mixture (Trottier et al., 1997). Therefore the amino acid mixture derived from mobilization of the body's muscle reserves is likely deficient in several essential amino acids and provides an excess of some non-essential amino acids, compared to that required for milk production and maintenance of mammary function. This conjecture is supported by our observations of changes in muscle free amino acid concentrations during lactation. When milk protein production fell in late lactation, intra-muscular concentrations of several free essential amino acids declined, suggesting the demand for these amino acids was in excess of supply. Whereas non-essential amino acids, especially glutamine, that are highly abundant in the mixture derived from muscle protein mobilization rose sharply, suggesting that they were in excess of supply (Chapter 4).

One can conceive of two alternative ways of meeting the sow's protein requirements in lactation, either by 1) mobilization of a large amount of the maternal protein mass generated in gestation, or 2) by provision of a high dietary protein intake during lactation. Based on our suggestion that the amino acid composition of mobilized endogenous protein does not match that required for milk production, we hypothesized that regardless of the sow's initial body protein mass, sows relatively more dependent on mobilization of endogenous reserves would express a poorer lactational and reproductive performance. This was not tested in our prior experiment because the sow's parturition protein mass was similar in all of the animals studied.

To test our hypothesis we fed pregnant gilts either 1) to industry standards to achieve a standard body (muscle) mass at parturition, or 2) to achieve a larger body (muscle) mass at parturition. In the subsequent lactation these sows were fed two different levels of protein to generate two levels of body protein mobilization. Litter growth and milk composition was monitored to assess the sow's lactational performance, and effects on the reproductive axis were measured as differences in the ovarian follicle population and follicular fluid estradiol (E_2) concentrations at weaning. Changes in the sow's protein and fat mass during gestation and lactation were measured indirectly from live-weight, backfat depth, and the prediction equations of Whittemore and Yang (1989). Body fat and muscle mass were determined after weaning, when animals were slaughtered.

5.2 MATERIALS AND METHODS

This experiment was conducted in accordance with the Canadian Council of Animal Care Guidelines, and was approved locally by the Institutional Animal Policy and Welfare Committee.

5.2.1 Experimental Treatments and Measurements

The experiment was conducted as a 2 x 2 factorial design in five replicates. The treatments consisted of feeding animals 1) in gestation to achieve two divergent body masses at parturition (high or standard), and 2) in lactation to achieve either a moderate (~10%) or high (~17%) level of maternal protein loss. Seventy-seven Genex gilts (Manor Hybrid x Large White or Manor Hybrid x Landrace; Genex Swine Group Inc.) were selected at a live-weight of 80 to 95 kg and placed into groups of five to seven animals in an environmentally controlled room. This study was not designed to measure differences among the sow genotypes, so to account for the inherent genetic differences due to the breed of the gilt's sire (Large White or Landrace), we randomized the two genotypes into the four treatments.

Gilts were heat checked daily until onset of standing estrus by placing an intact boar into the pen for 15 min/d. Gilts were bred two or three times, on at least their second estrus, by AI using pooled semen (Alberta Swine Genetics Corporation, Nisku, AB). In a few sows natural service was also used. Gilts were group-fed a conventional dry sow diet (12.1 MJ ME/kg, 13.3% CP, and 0.55% lysine) until 3 to 7 d after breeding. They were then individually penned and randomly allocated to their respective gestation feeding regimens to achieve either a standard gain of about 30 kg live-weight and 5 kg whole-body protein, or a high gain of about 65 kg live-weight and 10 kg whole-body protein by parturition. Gilt age (214 ± 1.2 d) and live-weight (128 ± 1.0 kg) at breeding were similar among treatments.

Gilts allocated to achieve a standard or high body mass at parturition were respectively fed 1.9 and 2.4 kg/d of the standard and high gain mash diet (Table 5-1). To maintain the desired gestational weight gains, individual sow feed intake was adjusted. Feed intake was increased in the standard and high body mass groups to be respectively 2.7 and 3.1

Table 5-1 Percentage composition (as-fed basis) of the gestation and lactation sow diets

	Gestation Diet[a]		Lactation Diet[b]	
Ingredient	Standard Gain	High Gain	High Loss[c]	Moderate Loss[d]
Wheat	24.0	43.9	27.0	27.0
Hulless Barley	-	-	34.4	34.4
Grinding Barley	62.9	23.8	-	-
Soybean Meal (46%)	7.0	23.0	16.0	16.0
Fishmeal (Herring)	-	-	-	8.0
Sugar	-	-	20.0	5.0
Oil (Canola)	2.0	5.0	5.0	5.0
Iodized salt	0.6	0.6	0.6	0.6
Dicalcium phosphate	2.2	1.8	2.4	2.0
Limestone	1.4	1.4	1.4	1.3
Choline Chloride (60%)	0.06	0.06	0.06	0.06
Hog Vit/Min Supplement [d]	0.20	0.20	0.20	0.20
Sow Vit/Min Supplement [d]	0.15	0.15	0.15	0.15
Lysine HCl	0.10	0.05	0.10	-
Valine	-	-	0.08	0.05
Calculated Analysis				
Energy, MJ ME/kg	12.86	14.18	14.34	14.03
Crude Fibre, %	4.69	3.66	2.76	3.24
Chemical Analysis				
DM, %	89.6	90.2	92.3	91.7
Crude Protein, %	14.00	18.17	10.51	16.61
Lysine, %	0.59	0.82	0.51	0.84
Valine, %	0.67	0.87	0.58	0.87

[a] Formulated to 0.91% Ca, 0.75% P

[b] Formulated to 0.93% Ca, 0.70% P

[c] Diet fed to sows during lactation to achieve a high degree of protein loss.

[d] Diet fed to sows during lactation to achieve a moderate degree of protein loss.

[e] The vitamin/mineral supplement supplied the following per kg of complete feed: 15,000 IU vitamin A, 1,500 IU vitamin D, 70 IU vitamin E, 2.5 mg vitamin K, 30 µg vitamin B_{12}, 9 mg riboflavin, 80 mg niacin, 30 mg calcium pantothenate, 1,200 mg choline chloride, 300 µg biotin, 2.9 mg folic acid, 3.0 mg pyridoxine; 5.0 mg thiamine; 200 mg iron, 90 mg manganese, 170 mg zinc, 25 mg copper, 1.0 mg iodine and 400 µg selenium.

kg/d from d 107 of gestation. These divergent energy (Head and Williams, 1991; Weldon et al., 1991) and protein intakes (Mahan and Mangan, 1975; Shields et al., 1985; Weldon et al., 1991; Kusina et al., 1999a) were not considered to either enhance or inhibit fetal and mammary gland growth and development. Approximately every 16 d during gestation sow live-weight was measured, and backfat depth was measured ultrasonically using an Aloka SSD-210DXII Echo camera with a UST-5020 diagnostic real-time ultrasound (Aloka Co. Ltd., Tokyo, Japan) equipped with a 110mm wide 3.5 Mhz probe head

(Overseas Monitor Corporation Ltd., Richmond, BC). Backfat was measured at three sites (loin site, mid-back, and grade site) as described by Sather et al. (1991) and modified (Chapter 6).

At approximately d 109 of gestation gilts were moved into individual crates in rooms containing five farrowing crates. At parturition sows (n = 53) were randomly allocated to achieve either a moderate or high level of protein loss. Sows were fed an isocaloric diet (~14 MJ ME/kg) containing divergent levels of CP (16.6 and 10.5%) and total lysine (0.84 and 0.51%) (Table 5-1) on an increasing scale. From d 1 of lactation sows were offered 3.0 kg/d, intake increased 1 kg/d every 5 d until d 15, and 5.5 kg/d was offered thereafter until weaning. To reduce the variance in feed intake between animals, feed levels were calculated to be approximately 85% of the ad libitum intake of first-parity sows in this herd. Feed intake was measured daily by weigh-back of feed refusals. Litter size was standardized to at least 9 pigs within 2 d of parturition by cross-fostering within the gestation feeding treatments. Routine procedures (teeth clipping, tail docking, ear-notching, and iron injection) were carried out 2 d postpartum, and no creep feed was offered to pigs in lactation. Sows and litters were weighed and sow backfat depth (mid-back) was measured ultrasonically on d 1 and every 5 d thereafter, including weaning. Additionally, litters were weighed on d 3, and sow backfat depth was measured ultrasonically at the three sites in early- (d 1 to 2) and mid-lactation (~d 15), and at weaning. Milk samples (10 to 20 mL) were obtained from sows, after an i.m. injection of 10 IU of oxytocin on d 10, and again on d 20 of lactation or 3 d prior to weaning, whichever came first. Milk samples were immediately stored at -20°C and later analyzed for protein, fat and lactose content.

Sows were weaned at about 0800 on d 25.6 ± 0.3, and within 2 to 3 h they were slaughtered. The evening prior to weaning feed was removed from the sows at 1800 to ensure that they had been fasted for at least 16 h prior to slaughter. A blood sample was collected into a 10 mL heparinized tube at exanguination, and stored on ice until processing. The blood sample was centrifuged at 1,500 x g for 15 min, and the plasma poured off and stored at -20°C for later insulin and IGF-1 analysis. The organs and other carcass variables were weighed (see below). Both ovaries were collected and washed twice in sterile saline containing kanamycin (0.1 mg/mL; Sigma, St Louis, MO). Ovarian function was assessed by measuring the size and number of follicles of > 2 mm diameter, follicular fluid volume, and follicle E_2 concentration and content.

5.2.2 Carcass measures and dissection (cut-out) of primal cuts

After slaughter and blood collection, the mammary gland was removed from the carcass, with the skin and connective tissue and some muscle attached, and weighed. The remaining hide was stripped from the carcass, removing as little subcutaneous fat as possible, and weighed. The viscera (kidneys, heart, kidney fat, spleen, full gut, and lungs, trachea, and tongue), and skinned head were removed and weighed. The liver was weighed after removal of the gall bladder, and the uterus was weighed after being trimmed of the mesenteric tissue, and sectioned immediately below the cervix. The carcasses were sawn in half and chilled for 24 h at 4°C after removal of the front feet, and the cold right half-side was cut into the primal cuts of the shoulder (picnic, hock and butt), loin, ham, and belly. The belly and side ribs were reduced to a trimmed and squared product. The remaining primal cuts (shoulder, loin, and ham) were separated into muscle, fat and bone, according to the boned-defatted cut-out procedure of Martin et al. (1981). The weights of these respective tissues were recorded, and the body cavity, subcutaneous, and inter-muscular fat depots for each cut were weighed separately and added together for the total fat in each depot. The muscle, fat and bone in the primal cuts were calculated to be twice the muscle, fat, or bone in the half-carcass primal cuts. Total carcass fat was calculated to be the fat in the carcass primal cuts plus the kidney fat.

5.2.3 Determination of sow body composition and energy and lysine balance

The measured variables of live-weight and backfat depth were used to estimate the sow's whole-body protein and fat mass using pre-existing equations (Whittemore and Yang, 1989). These equations were derived from proximate analysis of the weaned sow's carcass, and do not distinguish between skeletal muscle protein and that from other tissues and organs. A direct estimate of the size of the sow's relative muscle, bone and fat wet-weight at weaning was obtained by dissection (cut-out) of the primal cuts of the carcass at weaning as described above (Section 5.2.2).

Energy and lysine balance were calculated in lactation, based on the recorded measures of sow energy and total lysine intake minus the calculated requirements for sow maintenance and milk production. The maintenance requirements of the sow were assumed to be 106 kcal ME/kg body-weight $^{0.75}$ (444 kJ ME/ kg body-weight $^{0.75}$) (NRC 1998) and 0.039 g Lys/kg body weight $^{0.75}$ (0.049 g total Lys/kg body weight $^{0.75}$) (Fuller et al., 1989). The ME requirement for milk production ($\text{Energy}_{\text{milk}}$) was calculated from the equation of Noblet and Etienne (1989) that was modified by NRC (1998). The dietary efficiency of ME use for milk production was 72% (Noblet and Etienne, 1987). The total lysine requirement for milk production ($\text{Lysine}_{\text{milk}}$) was calculated from the equation of Pettigrew (1993).

$$\text{Energy}_{\text{milk}}\ (\text{kJ ME/d}) = [((4.92 \times \text{litter gain (g/d)}) - (90 \times \text{No. pigs}))/0.72] \times 4.184$$

$$\text{Lysine}_{\text{Milk}}\ (\text{g/d}) = 26 \times \text{litter gain (kg/d)}$$

5.2.4 Ovarian measures

The number of follicles > 2 mm diameter on both ovaries were recorded from each sow as an index of the size of the pre-ovulatory follicle pool. The external diameter of the follicle was determined by recording a mean of two calliper measurements taken at 90° to one another. Follicular fluid was individually aspirated from the largest eight follicles on each ovary, using a 250 μL Hamilton syringe, and collected. The weight of the syringe before and after aspiration was recorded, and the difference between these two weights was calculated as the follicular fluid weight. Follicular fluid volume was calculated assuming a density of 1 g/mL. Individual follicular fluid samples were diluted to 10% with tissue culture media (TCM 199 containing Earle's salts, L-glutamine and no sodium bicarbonate; GibcoBRL/Life Technologies, Grand Island, NY) and stored at -30°C in 1.5 mL micro-centrifuge tubes for later E_2 analysis. Peripherally acting E_2 effects were determined indirectly by assessing the stimulatory effects of E_2 on the weight of a target reproductive organ, the uterus.

5.2.5 Analyses

i) Feed and milk analyses. Feed samples were ground in a Wiley mill through a 0.8mm screen, mixed well, and stored at 4°C until DM, N, and amino acid analysis. Feed N was analyzed using the FP-428 Nitrogen Determinator, System: 601-700-900 (LECO Corporation, St. Joseph, MI). The amino acid composition of the feed was determined by HPLC (Sedgewick et al., 1991). Methionine, cysteine, tryptophan, and proline were not determined. The concentrations of milk fat, protein and lactose were determined by infrared analysis using a MilkoScan Analyzer (Foss Electrics, Denmark) at the Alberta Central Milk Testing Laboratory (Edmonton, AB).

ii) Plasma insulin and IGF-1 analyses. Plasma insulin concentrations were assayed by the double-antibody RIA described by Cosgrove et al. (1992). The sensitivity of the two assays was 0.019 ng/tube and the mean intra- and inter-assay CV were 5.6% and 11.9%. Plasma IGF-1 concentrations were determined after acid-ethanol extraction, as described by Cosgrove et al. (1992). Extraction efficiency, based on an estimate of cold recovery of IGF-1 added to the standard plasma pool, was 100%. The double-antibody RIA of Glimm et al. (1990) was used, with modifications described by Cosgrove et al. (1992). The assay sensitivity was defined as 92% of the total binding and the intra-assay CV was 11.2%.

iii) Follicular fluid E_2 analysis. Diluted follicular fluid (10% in TCM 199) from each sow was pooled into three categories based on volume; the four largest, four smallest and the four intermediate volumes. The pooled follicular fluid was further diluted 1:50 with PBS gel assay buffer ($NaH_2PO_4 \cdot H_2O$, 2.77 mM; NaH_2PO_4, 7.22 mM; NaCl, 139.7 mM; NaN_3, 15.38 mM; and 0.1% [w/v] gelatin; Sigma) to achieve a final dilution of 1:500. Estradiol concentrations were measured on these pooled samples using a Coat-A-Count RIA kit (Diagnostic Product Company, Los Angeles, California), with a minor modification. The kit Ab1 was diluted 3-fold in de-ionized H_2O to improve assay sensitivity, which was defined as 95% of total binding and was 0.03 ng/ml. The intra-assay CV, between 35 and 82% bound, for the two assays were 4.0 and 5.0% and the inter-assay CV was 6.9%.

iv) Statistical Analyses. All computations were performed using the GLM procedures of SAS (1990). Effects of the gestation feeding treatment on changes in sow live-weight and backfat depth were assessed using the repeated measures ANOVA. The model used was Y = Rep + BM + Br + BM x Br. Where Y = measured response, BM = gilt's body mass (standard or high) at parturition, Rep = number of replicates in the experiment ($n = 5$), and Br = breed of gilt's sire (Large White or Landrace). Effects of the lactation treatment on the sow and litter measures (actual and calculated) were assessed by repeated measures ANOVA. The model used was $Y = Rep + BM + PL + Br + BM \times PL + BM \times Br + PL \times Br + PL \times BM \times Br$. Where PL = lactation protein loss (high or moderate), and T = time. In the event of a significant T x BM or T x PL interaction, differences among Time within each treatment were computed using pre-defined orthogonal contrast. The sow, litter, carcass, and ovarian variables were assessed by ANOVA using the latter model. If significant treatment differences were detected ($P < 0.05$), these differences were computed using Fisher's protected least significant difference test.

5.3 RESULTS

Twenty-two of the 77 bred gilts placed on test were not pregnant and 2 others were taken off test in gestation because of lameness. Six of the 53 gilts that farrowed were taken off test because of illness and low appetite (≤ 3 kg/d in lactation). Twelve gilts were allocated to each treatment, except only 11 were available for allocation to the standard level of growth in gestation and to lose a high level of protein in lactation. Variation in the sow's genotype had no effect on most of the measured variables, except gilts with a Landrace sire had a higher ($P < 0.01$) litter growth rate throughout lactation. On the whole there were significant effects of the two main treatments, body mass at parturition and maternal protein loss in lactation, and few interactions among treatments. Any interactions are identified in tables as values without a common superscript letter.

5.3.1 Growth during gestation

Gilts fed divergent levels of CP (266 vs 436 g/d), total lysine (11 vs 20 g/d), and energy (24.4 vs 34.0 MJ ME/d) in gestation grew differentially. Gilts on the higher plane of nutrition achieved higher live-weight gains over the course of gestation (66 vs 41 ± 1.8 kg; $P < 0.001$), resulting in a larger (+17%) live-weight at parturition (193 vs 165 kg; $P < 0.01$). These sows also developed a larger ($P < 0.001$) skeletal structure, as indicated by about a 10% larger bone mass and a longer carcass (101 vs 96 cm) at weaning (Table 5-2). The belly, ribs and skinned head as well as the hide and most organs were also heavier

Table 5-2 Sow composition variables at parturition and weaning in first-parity sows fed to achieve a standard or high body mass at parturition

	Body Mass at Parturition[a]		
	Standard	High	Significance
Parturition:			
Live-weight, kg	165 ± 1.7[b]	193 ± 1.9	0.001
Backfat depth, mm	20.0 ± 0.9	22.7 ± 1.0	0.051
Estimated whole-body[c]:			
Protein mass, kg	24.3 ± 0.35	30.0 ± 0.36	0.001
Fat mass, kg	44.4 ± 1.50	54.5 ± 1.68	0.001
Protein:fat ratio	0.57 ± 0.017	0.56 ± 0.019	0.647
Carcass variables at weaning			
Muscle mass[d], kg	39.7 ± 0.96	48.0 ± 1.05	0.001
Fat mass[e], kg	15.4 ± 0.81	17.8 ± 0.89	0.048
Muscle:fat ratio	2.73 ± 0.14	2.89 ± 0.15	0.448
Bone[f], kg	8.63 ± 0.15	9.73 ± 0.16	0.001
Carcass length, cm	96.0 ± 0.84	100.7 ± 0.98	0.001
Skinned head, kg	4.76 ± 0.08	5.21 ± 0.08	0.001
Ribs, kg	3.78 ± 0.12	4.42 ± 0.14	0.002
Belly, kg	8.00 ± 0.36	9.72 ± 0.38	0.003
Hide, kg	12.2 ± 0.21	14.6 ± 0.24	0.001
Liver, kg	2.26 ± 0.05	2.47 ± 0.06	0.010
Heart, kg	0.48 ± 0.015	0.57 ± 0.017	0.001
Mammary gland[g], kg	7.32 ± 0.20	7.79 ± 0.22	0.124

[a] The lactation treatments were pooled within each gestation treatment (parturition body mass).

[b] Least-square mean ± standard error of the mean.

[c] Estimated whole-body protein /fat mass calculated using the equations of Whittemore and Yang (1989), based on sow live-weight and backfat depth.

[d] Muscle mass (kg) = 2 x half carcass muscle in the primal cuts (shoulder, loin and ham).

[e] Fat mass (kg) = kidney fat + (2 x half carcass fat in the primal cuts).

[f] Bone mass (kg) = 2 x half carcass bone in the primal cuts.

[g] Mammary gland weight, including attached skin and connective tissue and remnants of muscle.

($P < 0.01$) at weaning in the larger sows (Table 5-2). We did not slaughter animals at parturition for determination of body composition. However, when we analyzed muscle mass at weaning, we observed a significant residual effect of the gestational treatments. The magnitude of this effect (+20%; 48 vs 40 kg; $P < 0.001$) was concordant with the calculated estimate of whole-body protein mass at parturition which was about 23% higher in the larger sows (30.0 vs 24.3 kg; $P < 0.001$) (Table 5-2). Table 5-2 represents the effects of the two gestational treatments (parturition body mass). Because there were no interactions, the two lactational treatments were pooled within the gestational treatments.

The differences in muscle deposition during gestation were accompanied by parallel differences in fat deposition, such that at parturition the sow's muscle to fat ratio was the same after both feeding regimens. This was observed whether the values were estimated according to the equations of Whittemore and Yang (1989) (whole-body protein:fat ratio) or by carcass dissection at weaning (muscle weight : fat weight ratio) (Table 5-2). Backfat depth at parturition was not different between gestational feeding treatments. However, because of the animal's larger overall size, the calculated estimate of whole-body fat mass at parturition was larger (+23%; $P < 0.001$) in the larger sows. This was concordant with the larger (+15%; $P < 0.05$) amount of fat tissue dissected from the muscle tissue at weaning in the larger animals (Table 5-2).

Despite the differences in nutrient intake and animal growth in gestation, the feeding treatments imposed during gestation appeared to have little effect on mammary and fetal growth and development. Piglet birth weight (1.42 vs 1.35 ± 0.05 kg) and the number of pigs born alive (10.2 vs 11.0 ± 0.70) were similar among sows on the high and standard

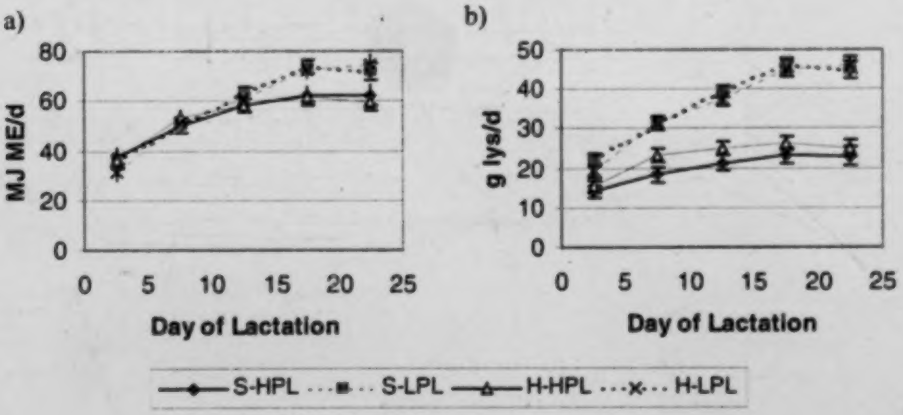

Figure 5-1 Intake of a) energy (MJ ME/d) and b) total lysine (g/d) in lactation by first-parity sows fed to achieve a standard (S) or high (H) body mass at parturition that lost a moderate (MPL) or high (HPL) amount of protein. Moderate protein loss sows had a higher ($P < 0.001$) total lysine intake throughout lactation. Energy intake was similar among treatments until d 10, and henceforth was slightly higher ($P < 0.001$) in moderate protein loss sows.

Table 5-3 Nutrient intake and sow live-weight and backfat changes over lactation in first-parity sows that had either a standard or high body mass at parturition and lost a moderate or high amount of protein during lactation

Parturition Mass	Standard Body Mass		High Body Mass		Significance[a]	
Lactation Loss	High	Moderate	High	Moderate	PBM	PL
Lactation intake:						
MJ ME/d	56.6 ± 2.3[b]	62.9 ± 2.2	55.9 ± 2.4	61.0 ± 2.3	0.590	0.016
CP, g/d	415 ± 22.8	743 ± 21.5	416 ± 23.8	720 ± 22.8	0.625	0.001
Total lysine, g/d	20.1 ± 1.3	37.7 ± 1.3	23.1 ± 1.4	36.2 ± 1.3	0.582	0.001
Sow live-weight						
at weaning, kg	141 ± 3.5	149 ± 3.3	163 ± 3.6	173 ± 3.5	0.003	0.013
Δ in lactation, kg	-23.1 ± 2.4	-17.6 ± 2.2	-29.7 ± 2.5	-21.3 ± 2.4	0.032	0.006
Δ in lactation, % d 0	-14.0 ± 1.39	-10.5 ± 1.31	-15.1 ± 1.44	-11.0 ± 1.38	0.569	0.008
Sow backfat depth[c]						
at weaning, mm	18.0 ± 1.3	16.5 ± 1.2	21.6 ± 1.3	17.2 ± 1.3	0.108	0.026
Δ in lactation, mm	-4.2 ± 1.0	-5.1 ± 0.9	-5.0 ± 1.0	-6.2 ± 1.0	0.330	0.288
Δ in lactation, % d 0	-18.0 ± 3.7	-22.6 ± 3.5	-18.5 ± 3.9	-24.6 ± 3.7	0.738	0.147

[a] Significance of effect of divergent parturition body masses (PBM) or lactational protein losses (PL).

[b] Least-square mean ± standard error of the mean.

[c] Average for all three sites.

planes of nutrition. The number of stillborn (0.53 ± 0.18) and mummified (0.21 ± 0.11) pigs, and the mammary gland wet weight determined at weaning also did not differ between gestation treatments (Table 5-2).

5.3.2 *Lactational treatments*

Lactational energy and lysine intake increased ($P < 0.001$) in all treatments until d 20, and increased no further thereafter (Figure 5-1ab); similar changes were observed for CP intakes. Sows fed to lose a moderate amount of protein had higher ($P < 0.001$) protein (416 vs 731 g/d) and total lysine (21.6 vs 37.0 g/d) intakes during lactation than sows fed to lose a high amount of protein (Table 5-3). Energy intake did not differ among lactation treatments over the first 10 d of lactation, but thereafter were about 10% higher ($P < 0.05$) in sows fed to lose a moderate rather than a high amount of protein (Figure 5-1a). Litter size on d 0 and 3 of lactation and at weaning were similar among treatments and were respectively 10.7, 10.3, and 9.9 ± 0.22 piglets.

5.3.3 *Litter performance*

Litter growth rate increased ($P < 0.001$) over the first 10 d of lactation for sows on all

Table 5-4 Litter measures in first-parity sows that had a standard or high body mass at parturition and lost a moderate or high amount of protein during lactation

Parturition Mass	Standard Body Mass		High Body Mass		Significance[a]	
Lean Loss	High	Moderate	High	Moderate	PBM	PL
Total litter gain, kg	49.9 ± 2.4[b]	56.1 ± 2.2	53.7 ± 2.5	57.7 ± 2.4	0.261	0.033
Mammary gland[c]	6.96 ± 0.29	7.68 ± 0.27	7.41 ± 0.30	8.16 ± 0.29	0.124	0.014
Litter gain, kg/d:						
d 10 to 15	2.27 ± 0.10	2.41 ± 0.10	2.37 ± 0.11	2.56 ± 0.10	0.221	0.103
d 15 to 20	2.11 ± 0.12	2.41 ± 0.11	2.35 ± 0.12	2.66 ± 0.12	0.044	0.011
d 20 to 26	1.76 ± 0.16	2.21 ± 0.15	1.83 ± 0.17	2.37 ± 0.16	0.485	0.004
Δ litter growth rate, % d 10 to 15 vs d 20 to 26	-19.5 ± 6.9	-6.8 ± 6.5	-21.6 ± 7.2	-6.4 ± 6.9	0.908	0.045

[a] Significance of effect of divergent parturition body masses (PBM) or lactational protein losses (PL).

[b] Least-square mean ± standard error of the mean.

[c] Mammary weight (wean), including attached skin, connective tissue and muscle remnants.

treatments, and thereafter remained unchanged until d 20, when a decline in litter growth was observed (Figure 5-2). The most catabolic sows, regardless of their parturition body mass, had several difficulties in maintaining performance at the end of lactation. Litter growth rate started to decline earlier (Figure 5-2a; $P < 0.05$), the decline was larger (Table 5-4; $P < 0.05$), and overall litter growth rate was lower in these sows (Table 5-4; $P < 0.05$). Milk protein concentration was also lower in the most catabolic sows (Figure 5-3a;

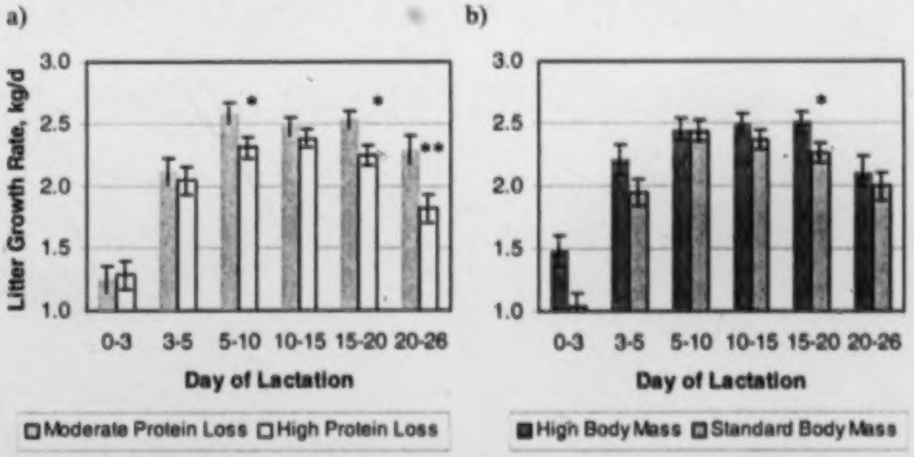

Figure 5-2 Litter growth rate (kg/d) of first-parity sows that a) lost a moderate or high amount of protein during lactation, and b) had either a high or standard body mass at parturition. Symbols denote that treatments within the same time period differ: * = $P < 0.05$, ** = $P < 0.01$.

$P < 0.05$), but milk fat and lactose concentrations did not differ among treatments. Milk fat concentrations did however decline between d 10 and 20 of lactation (Figure 5-3b; $P < 0.001$). The poor piglet performance observed at the end of lactation may have been related to alterations in the mammary gland, which had about a 10% smaller mass at weaning in the most catabolic sows (Table 5-4; $P < 0.02$).

A smaller body mass at parturition seemed to exacerbate the effects of restricted feeding in lactation, as the decline in litter growth rate appeared to occur earlier in the smaller sows (Figure 5-2b; $P < 0.02$). Overall litter weight gain was similar for sows fed to achieve a standard and a large body mass at parturition (53.0 vs 55.7 ± 1.70 kg; $P = 0.261$) (Table 5-4). The small difference between these two treatments was largely attributable to a lower litter growth rate over the first 3 d of lactation in the smaller sows (Figure 5-2b; $P < 0.01$). However, large and small sows did not differ in the degree of decline of litter growth in late lactation (Table 5-4).

5.3.4 *How catabolic were the sows in lactation*

The live-weight losses in lactation and body weights at weaning reflected the level of protein feeding during lactation and the size of the sow's body mass at parturition (Table 5-3). Sows fed the lower protein level during lactation lost a larger fraction ($P < 0.01$) of

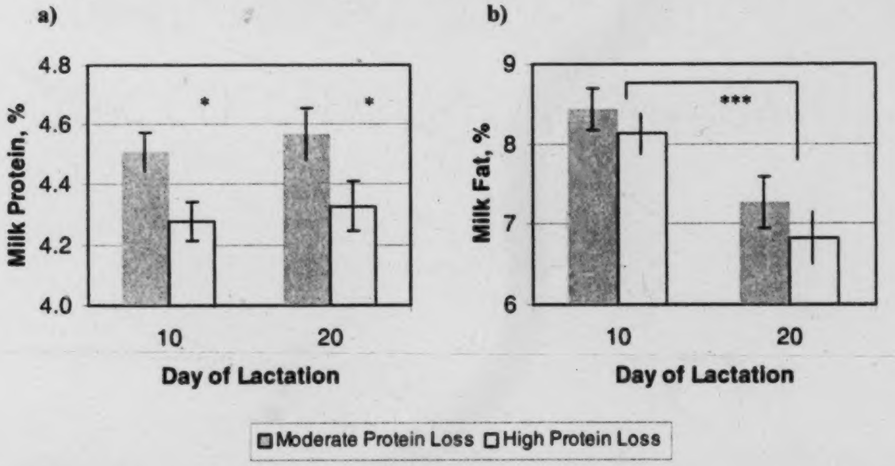

Figure 5-3. Milk a) protein and b) fat composition on d 10 and 20 of lactation in first-parity sows that lost a moderate or high amount of protein during lactation. Symbols (*) denote that treatments within the same time period differ ($P < 0.05$), or that variables decline between d 10 and 20 of lactation (*** = $P < 0.001$).

Table 5-5 Calculated energy and lysine balance and change in whole-body protein and fat mass over lactation in first-parity sows that had a standard or high body mass at parturition and lost a moderate or high amount of protein during lactation

Parturition Mass	Standard Body Mass		High Body Mass		Significance[a]	
Lactation Loss	High	Moderate	High	Moderate	PBM	PL
Estimated cumulative whole-body protein loss[b], % parturition mass:						
to d 15	-9.8 ± 1.5[c]	-4.3 ± 1.4	-9.6 ± 1.6	-7.1 ± 1.5	0.402	0.012
to d 20	-12.5 ± 1.5	-3.4 ± 1.4	-12.4 ± 1.5	-7.3 ± 1.5	0.195	0.001
to weaning	-17.3 ± 1.6	-10.6 ± 1.5	-16.7 ± 1.7	-11.2 ± 1.6	0.991	0.001
Estimated cumulative whole-body fat loss[b], % parturition mass:						
to d 15	-14.6 ± 2.2	-12.6 ± 2.1	-16.9 ± 2.3	-16.4 ± 2.2	0.189	0.577
to d 20	-19.8 ± 2.6	-21.7 ± 2.4	-20.1 ± 2.7	-20.2 ± 2.6	0.822	0.702
to weaning	-23.0 ± 3.7	-25.0 ± 3.2	-24.5 ± 3.5	-23.9 ± 3.4	0.950	0.844
Calculated balances in overall lactation						
Energy, MJ ME/d	-16.9 ± 3.0	-20.4 ± 2.7	-23.2 ± 2.8	-25.7 ± 2.9	0.053	0.298
Lysine, g/d	-23.4 ± 1.9	-16.8 ± 1.7	-23.6 ± 1.7	-19.1 ± 1.8	0.500	0.003

[a] Significance of the divergent parturition body masses (PBM) or lactational protein losses (PL).
[b] Whole-body protein/fat mass calculated from the equations of Whittemore and Yang (1989).
[c] Least-square mean ± standard error of the mean.

their parturition live-weight (-14.5 vs -11%; Table 5-3) and estimated whole-body protein mass (-17.8 vs -10.7%; Table 5-5). This was concordant with the approximately 10% lower ($P < 0.001$) muscle mass and estimated whole-body protein mass at weaning in sows fed the lower protein level (Table 5-6). The most catabolic sows also had lighter organs (kidneys, heart and liver) and hide ($P < 0.01$) weights at weaning (Table 5-6). The smaller sows had a smaller body-weight (140 vs 163 kg; $P < 0.05$; Table 5-3), muscle mass (39.7 vs 48.0; $P < 0.005$) and calculated whole-body protein mass (20.8 vs 24.9 kg; $P < 0.001$) at weaning (Table 5-6). This resulted in the small sows that lost the most protein in lactation having the lowest relative muscle mass at weaning; these sows had a lower ($P < 0.01$) muscle to bone ratio at weaning compared to the other three treatments (4.25 vs 5.0; Table 5-6).

During lactation sows on all four treatments lost backfat depth (-5mm; Table 5-3) and estimated whole-body fat mass (approximately -24% parturition fat mass; Table 5-5). The calculated energy balance (-22.3 ± 2.4 MJ ME/d) was also negative and did not differ among treatments (Table 5-5). However, sows fed the lower protein level in lactation lost slightly less fat tissue in lactation. At weaning these sows had a deeper backfat depth

Table 5-6 Measured carcass variables and plasma hormone concentrations, and calculated carcass variables at weaning in first-parity sows that had a standard or high body mass at parturition and lost a moderate or high amount of protein during lactation

Parturition Mass	Standard Body Mass		High Body Mass		Significance^{a}	
Lactation Loss	High	Moderate	High	Moderate	PBM	PL
Muscle mass^{b}, kg	37.3 ± 1.4^{c}	42.1 ± 1.3	46.1 ± 1.5	49.9 ± 1.4	0.001	0.003
Whole-body protein^{d}, kg	19.9 ± 0.63	21.7±0.60	23.6±0.66	26.1 ±0.63	0.001	0.001
Muscle to bone ratio	4.25±0.16^{x}	5.00±0.15^{y}	4.89±0.16^{y}	5.01 ±0.16^{y}	0.052	0.007
Fat mass^{e}, kg	14.5 ± 1.05	12.9±0.99	17.1 ± 1.09	14.9 ± 1.05	0.036	0.064
Kidney fat	1.90 ± 0.19	1.47±0.18	2.02 ± 0.20	1.64 ± 0.19	0.474	0.036
Total carcass fat ^{f}, kg	16.4 ± 1.2	14.3 ± 1.1	19.2 ± 1.2	16.5 ± 1.2	0.048	0.048
Whole-body fat ^{d}, kg	32.7 ± 2.1	32.5±1.9	42.6 ± 2.1	38.4 ± 2.1	0.001	0.278
Other carcass tissues and organs, kg:						
Hide	11.6 ± 0.31	12.9±0.29	14.3 ± 0.33	14.9 ± 0.31	0.001	0.003
Full gut	10.2 ± 0.46	11.0±0.43	10.5 ± 0.48	12.1 ± 0.46	0.132	0.011
Lungs^{g}	1.99±0.08^{x}	2.07±0.08^{x}	2.05±0.08^{x}	2.45±0.08^{y}	0.010	0.004
Kidneys	0.39±0.02^{x}	0.44±0.01^{y}	0.40±0.02^{xy}	0.52±0.02^{z}	0.001	0.001
Heart	0.47 ± 0.02	0.50±0.02	0.53 ± 0.02	0.62 ± 0.02	0.001	0.009
Liver	2.11 ± 0.08	2.40±0.07	2.22 ± 0.08	2.73 ± 0.08	0.010	0.001
Spleen	0.28 ± 0.04	0.31±0.04	0.27 ± 0.04	0.37 ± 0.04	0.546	0.110
Pre-prandial plasma hormone levels:						
IGF-1, ng/ml	54.9 ± 4.6	57.4±4.5	59.7 ± 4.8	66.2 ± 4.6	0.157	0.322
Insulin, pg/ml	337 ± 37	364 ± 37	370 ± 38	461 ± 39	0.091	0.124

[a] Significance of effect of divergent parturition body masses (PBM) or lactational protein losses (PL).

[b] Muscle mass (kg) = 2 x half carcass muscle in the primal cuts (shoulder, loin and ham).

[c] Least-square mean ± standard error of the mean.

[d] Whole-body protein/fat mass calculated from the equations of Whittemore and Yang (1989).

[e] Fat mass (kg) = 2 x half carcass fat in the primal cuts (shoulder, loin and ham).

[f] Total carcass fat (kg) = kidney fat + fat mass.

[g] Weight of lungs, trachea and tongue.

[xyz] Within a row, means without a common superscript letter differ ($P < 0.05$).

(21.6 vs 17.2mm; $P < 0.05$; Table 5-3), and their carcass contained more kidney fat and total fat mass (Table 5-6; $P < 0.05$).

5.3.5 *Ovarian Function*

Only ovarian function data from the last four replicates are presented here because not all the ovarian variables were measured in sows from the first replicate. Furthermore, because the follicular fluid variables from the different sized follicle pools (the smallest, moderate and largest four follicles) did not differ among treatments, only the average value for these variables is presented.

Table 5-7 Ovarian measures at weaning in first-parity sows that had a standard or high body mass at parturition and lost a moderate or high amount of protein during lactation

Parturition Mass	Standard Body Mass		High Body Mass		Significance[a]	
Lean Loss	High	Moderate	High	Moderate	PBM	PL
n	6	10	8	8		
Ovarian follicle No.:						
≥ 2mm diameter	34 ± 7.0$^{b\,x}$	63 ± 6.4^{y}	65 ± 7.6^{y}	61 ± 7.5^{y}	0.045	0.080
> 3.5mm diameter	4.9 ± 2.7	8.8 ± 2.5	11.7 ± 3.0	15.0 ± 2.9	0.025	0.195
Largest 16 follicles:						
Diameter, mm	3.2 ± 0.33	3.3 ± 0.29	3.9 ± 0.29	4.0 ± 0.27	0.025	0.842
FF volume, µl	23.0 ± 4.6	20.7 ± 4.0	31.2 ± 3.9	34.1 ± 3.8	0.017	0.939
FF E_2, ng/ml	0.22 ± 0.13^{v}	0.17 ± 0.11^{v}	0.33 ± 0.11^{v}	0.67 ± 0.11^{w}	0.015	0.195
FF E_2, ng/follicle	5.0 ± 5.2^{v}	3.6 ± 4.6^{v}	9.6 ± 4.4^{v}	25.4 ± 4.4^{w}	0.011	0.130
Uterine weight[c], kg	0.24 ± 0.02^{x}	0.22 ± 0.02^{x}	0.23 ± 0.02^{x}	0.30 ± 0.02^{y}	0.060	0.126

[a] Significance of effect of divergent parturition body masses (PBM) or lactational protein losses (PL).

[b] Least-square mean ± standard error of the mean.

[c] Uterine weight at weaning from all sows, trimmed of excess connective tissue.

[xy] Within a row, means without a common superscript letter differ ($P < 0.05$)

[vw] Within a row, means without a common superscript letter differ ($P < 0.10$)

The size of the sow's body mass at parturition had the most overriding effect on the ovarian variables measured (Table 5-7; $P < 0.05$). Of the large sows, those that were the least catabolic tended ($P < 0.08$) to have double the follicular fluid E_2 concentration and content, had the highest ($P < 0.05$) uterine weight at weaning, indicating higher peripheral E_2 concentrations (Table 5-7). They also tended ($P < 0.10$) to have higher pre-prandial plasma insulin concentrations at weaning compared to the other three treatments (461 vs ~360 pg/ml), but similar pre-prandial plasma IGF-1 concentrations (Table 5-6).

The sows that had the lowest muscle mass (37.3 vs 42.1, 46.1 and 49.9 kg), estimated whole-body protein mass (19.9 vs 21.7, 23.6, and 26.1 kg), and muscle to bone ratio (4.3 vs 5.0, 4.9, and 5.0) at weaning had the lowest ovarian function. These sows had about 50% fewer follicles (≥ 2mm diameter) compared to the other three treatments (Table 5-7; $P < 0.05$). Also the ovaries of almost 20% of these sows (2 out of 11) appeared to have almost completely "shut-down" at weaning; the ovaries of these sows contained follicles of less than 2mm diameter.

5.4 DISCUSSION

In this experiment, gilts in gestation were fed to either industry standards or to a higher level (> 50% more energy, protein and lysine) which resulted in sows of different live-weights (165 vs 193 kg) but similar body composition at parturition (protein to fat ratio = 0.57). Animals on the different gestation treatments therefore had divergent estimated whole-body protein masses (24.3 vs 30.0 kg) to draw upon in lactation. This gestational feeding regimen, despite causing divergent growth had no apparent affect on mammary growth and development, or on fetal growth and development apparent at birth or during lactation.

By feeding sows in lactation an iso-energetic diet (~14 MJ ME/kg) that contained divergent levels of protein and lysine, sows lost differential proportions of their estimated whole-body protein mass and live-weight. Litter growth rate did not differ between lactation treatments when the small (10%) divergence in energy intake was first observed (d 10 to 15). This suggests that the difference in sow energy intake between lactation treatments was insufficient to limit milk synthesis; energy intake is thought to be the primary controller of milk production in the pig (Tokach et al., 1992). The difference in energy intake also did not alter the sow's calculated energy balance.

A decline in litter growth rate occurred upon loss of 10 to 12% of the sow's estimated initial (parturition) whole-body protein mass, independent of the size of the sow's body (muscle) mass. This agrees with our previous experiment (Chapter 3), in which a decline in litter growth rate occurred upon mobilization of a similar amount of the sow's estimated whole-body protein mass. A decline in several ovarian variables (index of reproductive performance) also occurred in this and the prior study, with an onset and progressive decline that mirrored the decline in lactational performance. Overall the poorest lactational performance and measured ovarian variables were seen in animals that were initially smaller and the most catabolic during lactation. A larger body mass at parturition therefore delayed attainment of the decline in litter growth (occurred ~5 d earlier in smaller sows) and ovarian variables.

5.4.1 Impact of varying degrees of protein loss on lactational performance

A number of mechanisms are conceivably involved in the decline in lactational performance observed in sows that lost large amounts of their muscle protein mass. The first is simply based on the idea that, in animals that mobilize protein in lactation to maintain milk production, the sow's muscle mass progressively declines as lactation continues. To provide a consistent supply of amino acids to provide substrates for milk production, the fractional rate of muscle protein mobilization must increase by a similar degree. However, there appears to be an upper limit to the fractional rate of muscle protein mobilization (Chapter 3). With continuous loss of body protein throughout lactation, at some point this maximal fractional rate of muscle protein mobilization will no longer be sufficient to supply all the amino acids required to maintain milk production, and as a result milk production, and therefore litter growth rate, would fall.

Other possible mechanisms that cause the reduction in litter growth, and by inference milk production, observed in this experiment, relate to our previous observation that sow's more dependent on their endogenous protein reserves in lactation are likely to be deficient in some essential amino acids (Chapter 4). In both the lactating pig and dairy goat, milk yield is positively related to the arterio-venous differences of essential amino acids across the gland, and the rate of blood flow through the gland (Bequette et al., 1997; Trottier et al., 1997). Further evidence that nutrient uptake by the mammary gland is related to its supply of essential amino acids are provided by Metcalf et al. (1994, 1996). These authors observed increased arterio-venous differences of phenylalanine across the mammary gland upon short-term (4 h) phenylalanine infusion to lactating dairy cows (22 wk postpartum) with a tendency for an increased milk yield (Metcalf et al., 1994). The same study observed an increase (+9%; $P < 0.05$) in milk yield upon infusion of methionine. Similarly, increasing the intake of rumen-protected protein by lactating dairy cows increased the arterial supply of essential amino acids to the mammary gland and increased milk production and milk protein yield and concentration (Metcalf et al., 1996). But the supply and mammary uptake of non-essential amino acids was unaffected.

The amino acid mixture released upon mobilization of endogenous (muscle) protein reserves does not match that required by the lactating mammary gland (Chapter 4). This mixture is low in several essential amino acids required at high levels by the gland, and is more than replete in some non-essential amino acids, especially glutamine, and causes similar alterations in the muscle free amino acid pool (Chapter 4). Muscle is the body's main source of free amino acids, and changes here are reflected in the plasma pool that supplies the needs of the mammary gland (Chapter 1). The presence of high peripheral non-essential amino acids concentrations, such as glutamine, may inhibit the uptake of essential amino acids by the mammary gland because of competition for use of the various mammary gland transporter systems. Glutamine and alanine inhibit lysine uptake by rat mammary tissue explants (Shennan et al., 1994) and valine uptake in porcine mammary tissue explants (Jackson et al., 2000). Physiological concentrations of arginine and ornithine and supraphysiological concentrations of leucine, methionine and alanine inhibited lysine uptake by porcine mammary tissue explants (Hurley et al., 2000). Methionine, leucine, and lysine also inhibited valine uptake in porcine mammary tissue explants (Jackson et al., 2000).

An induced deficiency of a single essential amino acid (histidine) in the lactating goat reduced milk protein output by about 20%, despite an approximately 33% increase in mammary blood flow (Bequette et al., 2000). Similarly a large imbalance in the amino acid intake of lactating dairy cows, by reducing the supply of abomasal methionine, lysine or histidine, reduced the yield of milk protein by 15%, but increased milk fat yield by almost 30% (Weekes and Cant, 2000). The removal of abomasal histidine had the most dramatic effect on milk protein and fat output followed by lysine and methionine. In the same study, a reduction in the abomasal supply of BCAA's had no effect on milk protein and fat yield. Thus a highly imbalanced mammary amino acid supply could inhibit milk protein synthesis prior to a depression milk production. This may explain the depression in milk protein concentration observed in this experiment, prior to the decline in litter growth rate, in sows fed low levels of total lysine (22 vs 37 g/d). A similar reduction in milk protein concentrations in first-parity sows fed low lysine intakes in lactation (10 vs 30 g/d) was observed by Kusina et al. (1999b) as early as d 8 of lactation, and was still apparent on d 18.

The lower litter growth rate, and by inference milk production, observed in sows fed the lower protein intake in lactation resulted in a slightly lower (~10%) energy expenditure in these sows for milk production, especially at the end of lactation. This likely reduced the need of these animals to mobilize their adipose tissue reserves, and as a result they had a slightly higher fat mass (17.8 vs 15.4 kg), kidney fat mass (1.96 vs 1.54 kg) and backfat depth (19.8 vs. 16.8mm) at weaning. Other authors also observed that first-parity sows fed similar energy but higher lysine levels in lactation lost more body protein but lost less backfat (Brendemuhl et al., 1989; King et al., 1993; Everts and Dekker, 1994; Sauber et al., 1998) and kidney fat in lactation (Sauber et al., 1998).

5.4.2 Impact of varying degrees of protein loss on ovarian function

Inadequate nutrition, and more specifically inadequate protein intakes, in lactation can impair the reproductive axis. The nutritionally-induced inhibition of the reproductive axis may be observed at the level of the hypothalamic-pituitary axis by a decline in pulsatile LH release from the anterior pituitary, due to inhibition of the hypothalamic GnRH pulse generator (Booth, 1990; I'Anson et al., 1991; Wade and Schneider, 1992, 1996). In late lactation and after weaning, LH pulsatility was reduced in sows that were either restrict-fed (Zak et al., 1997a; Quesnel et al., 1998; van den Brand et al., 2000) or protein-restricted in lactation (King and Martin, 1989; Jones and Stahly, 1999b; Yang et al., 2000b). This, together with nutritionally-induced factors such as changes in substrates and hormonal levels, directly inhibit ovarian function and ovarian follicle growth and development, and the quality of the follicle and oocyte within that follicle (Foxcroft, 1990, 1992). Antral follicles may be recruited into the pre-ovulatory pool in the span of 19 to 21 d (Morbeck et al., 1992). Because the lactation length of sows in this experiment was 26 d, follicles that constitute the pre-ovulatory pool were probably undergoing antral formation in early lactation, and could be affected by the nutritional state of the sow at any stage of lactation.

The impairment of follicle development in large sows that were fed the lower rather than the higher protein level, and small sows that were fed a lower rather than a higher protein intake in lactation, may be explained by the difference in the sow's protein nutrition. Other researchers also observed that first-parity sows restricted in protein intake during lactation have a smaller preovulatory follicle pool (> 3mm diameter) at weaning (Chapter 3), and poorer quality follicles after weaning (Zak et al., 1997b; Yang et al., 2000a;

Chapter 3). Similarly, restrict-fed sows in lactation had fewer preovulatory follicles at weaning (Quesnel et al., 1998), and poorer quality follicles (Zak et al., 1997b) and lower ovulation rates after weaning (Zak et al., 1997a; van den Brand et al., 2000).

Body size, and therefore the size of the sow's muscle protein reserves, had the most overriding effect on ovarian variables at weaning, with the larger sows having the highest ovarian fun[illegible]n. Although, the estimated amount of parturient whole-body protein mass lost by the most and least catabolic animals in lactation did not differ with body size (17 vs 11%). When only the loss of muscle protein was taken into consideration, sows that lost the largest amount of muscle protein had the lowest ovarian function at weaning. This was confirmed in Chapter 6. These sows had a small body mass and were fed the lower protein intake in lactation. Small sows had about a 20% smaller estimated whole-body protein mass at parturition compared to the larger sows. The small sows that were most catabolic had a lower relative muscle mass at weaning, as indicated by a smaller muscle to bone ratio compared to the other treatments. The muscle to bone ratio can be used as an index of the relative muscle mass of post-pubertal animals because bone mass is maintained, and is not mobilized to any great degree, even under conditions of relatively extreme weight loss in the adult animal. Bone mass did not differ in steers (initial weight 390 kg) that lost between 0 and 20% of their live-weight (Seebeck, 1973), and the lean to bone ratio was similar in steers of 18 and 24 months of age with divergent live-weights (215 vs 265 kg) (Kempster, 1978).

Achievement of a low muscle mass in the body (lower critical muscle mass) probably initiates warning signals that inhibit the reproductive axis to delay or even prevent the subsequent reproductive cycle to allow the animal to recover from the large metabolic insult. Peripheral insulin and IGF-1 concentrations likely did not mediate this inhibition of the reproductive axis, because in this and our previous experiment (Chapter 3), peripheral levels of these hormones did not differ among treatments. However, changes in insulin and IGF-1 sensitivity could have occurred at the tissue level due to changes in variables such as receptor number, affinity of ligand binding, and signal transduction. Such changes in hormonal sensitivity could alter the signal transmitted by the circulating hormonal levels to affect the hypothalamo-pituitary-ovarian axis either directly or indirectly. It is conceivable that the peripheral amino acid patterns could be altered, as described above, to: 1) directly impair ovarian function, and 2) inhibit the hypothalamic-pituitary axis by altering concentrations of neurotransmitters; as some amino acids can act directly as, or are precursors for, neurotransmitters.

5.4.3 Changes in non-mammary tissues in lactation

Skeletal muscle, like many other tissues, changes its metabolism in lactation to increase the proportion of amino acids and other substrates supplied to the mammary gland for milk biosynthesis. Various mechanisms may be involved in the improvement of substrate availability to the mammary gland in lactation, and include decreasing the utilization of amino acids by extra-mammary tissues such as the skin and liver, and/or increasing the mobilization of amino acids from tissues such as muscle. This latter point is discussed in more detail in the following chapter (Chapter 6). Liver protein concentrations do not change in lactation as a response to nutritional deprivation (Chapter 2), and the fractional rate of liver protein synthesis does not change with physiological state (e.g. lactating vs gestating animals; Champredon et al., 1990; Baracos et al., 1991), or protein nutrition (Pine et al. 1994ab). Therefore, a reduction in liver mass reflects a decrease in both liver protein mass and the absolute rate of liver protein synthesis. The liver adapts to lactation by excreting less nitrogen in urine as lactation progresses, reflecting a decreased hepatic amino acid metabolism (Noblet and Etienne, 1987; Chapter 2). As lactation progresses the liver further adapts to lower protein intakes in lactation, by further reducing the urinary N output per unit of dietary protein absorbed. Thus the smaller livers at weaning in sows that mobilize a large amount of protein in lactation, indicates these animals have a reduced absolute rate of hepatic protein synthesis (Brendemuhl et al., 1989; Sauber et al., 1998; Chapter 3). The lower organs weights (e.g. heart and kidneys) at weaning in sows fed low lactational protein intakes, in this and similar experiments (Brendemuhl et al., 1989; Sauber et al., 1998), indicates that these organs also adapt in lactation to allow additional nutrients to be diverted to the mammary gland.

5.4.4 Conclusion

This experiment confirms our hypothesis that first-parity sows that are more dependent on mobilizing their endogenous protein reserves, express a poorer lactational and reproductive performance, independent of their body size at parturition. These data also support the presence of a critical amount of protein loss, above which animal performance declines (estimated to be 10 to 12% of the sow's parturition whole-body protein mass). The larger body protein reserve in the bigger animals appears to delay the decline of at least some functions, such as litter growth and possibly the impairment of ovarian function. The poorest overall lactational performance and measured ovarian variables were observed in the most catabolic animals that had a smaller initial body (muscle) mass. These sows also had the lowest relative muscle mass at weaning (muscle to bone ratio), which supports the idea of a lower critical muscle protein mass.

The decline in litter growth was probably caused by a reduced uptake of essential amino acids by the mammary gland, caused by a number of factors. These probably include first a reduced peripheral concentration of some essential amino acids. Second, an increased competition for the uptake of these amino acids by the mammary gland because of the elevated levels of some non-essential amino acids e.g. glutamine. Third, a muscle protein mass that is too small, so that the maximal fractional rate of muscle protein mobilization is unable to provide all the amino acids required for mammary function and to maintain milk protein synthesis. All these factors likely contribute to a deficiency in essential amino acid uptake by the mammary gland and ultimately to the reduction in milk protein synthesis and milk production observed. The decline in reproductive performance could be initiated by the achievement of a smaller muscle mass that sends warning signals to inhibit the reproductive axis and delay or even prevent the subsequent reproductive cycle.

5.4 APPENDIX: The impact of gestational feeding on post-weaning progeny growth

The progeny of sows on the two gestation feeding treatments reached slaughter weight (107 kg) at the same age (158 d). However, the progeny of sows fed the high gestation feeding regimen were leaner; and had a lower (-10%; $P < 0.01$) backfat depth and a higher (62.1 vs 61.2; $P < 0.01$) predicted saleable meat yield at slaughter (Fortin et al., 2000). This suggests that these animals may have developed more secondary muscle fibres either in utero, or in the first few days of life when their growth was about 30% higher than the progeny of sows fed the standard gestation treatment. The number of secondary muscle fibres in the progeny can be increased by about 10%, by increasing the sow's feed intake (5.0 vs 2.5 kg/d) in early gestation (d 25 to 50) (Dwyer et al., 1994). Conversely, under-nutrition in gestation inhibits fetal muscle development and fibre number in the guinea-pig (Dwyer and Stickland, 1994; Dwyer et al., 1995) and sheep (McCoard et al., 1997). Mild postnatal under-nutrition also appears to influence muscle development at the molecular and functional level in the weaned pig (White et al., 2000). To further address this issue and confirm this theory, more research focusing on the effect of gestational nutrition on the progeny's muscle fibre number, and their muscle growth and development is required.

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CHAPTER SIX

Evaluation of mobilization of maternal muscle protein mass during lactation

6.1 INTRODUCTION

Skeletal muscle is the main reserve of body protein accessible for mobilization during nutritional, physiological and pathological states where protein requirements exceed the dietary supply (Allison et al., 1963; Allison and Wannemacher, 1965; Swick and Benevenga, 1977). Protein synthesis and catabolism in skeletal muscle are highly regulated, and sensitive to indices of nutritional status such as starvation (Lowell et al., 1986; Li and Wassner, 1984) and inadequate dietary intake in lactation (Tesseraud et al., 1993; Pine et al., 1994ab), in a manner that permits rapid protein mobilization. In lactating animals, the presence of a protein reserve ensures that milk production is maintained under conditions of poor nutrient supply (Pluske et al., 1998). However, significant erosion of this reserve is associated with a decline in milk production and litter growth (Kusina et al., 1999; Jones and Stahly, 1999a; Chapters 3 and 5) and in subsequent reproductive performance (King, 1987; King and Martin, 1989; Jones and Stahly, 1999b; Chapters 3 and 5).

Measures of muscle protein mass are an essential component of research into 1) the recruitment of maternal protein for maintenance of animal performance, and 2) the impact of excessive muscle protein mobilization on animal performance. Preferably these measures should be non-invasive so that determinations can be made in real-time over the course of the progressive muscle protein loss. Several approaches are available and have been used to assess body protein changes in lactating sows. Nitrogen balance has been used in the lactating sow to predict changes in the sow's whole-body protein mass (Noblet et al. 1987; King et al., 1993; Dourmad et al., 1998). However the inherent inaccuracies of this method (Just et al., 1982; Manatt and Garcia, 1992) make it a poor predictor of large changes in body protein mass and in changes of body protein mass over long periods (over three weeks) (Everts and Dekker, 1994; Dourmad et al., 1998). Also N losses in milk can only be indirectly assessed in the pig, and urinary tract catheterization is required, increasing the potential for introduction of infection into the sow. The comparative slaughter technique measures the animal's body composition directly after slaughter, by dissection and chemical determination of the various constituents (protein and fat). This approach is clearly the most direct, but is expensive, requires specialized equipment, repeated measures cannot be made in the same animals and is very time consuming. From these comparative slaughter measures, equations have been formulated to estimate the sow's whole-body protein and fat mass based on deuterium oxide dilution (Shields et al., 1984) and non-invasive measures such as body weight and backfat thickness (Whittemore and Yang, 1989; Mullan and Williams, 1990; Everts and Dekker, 1995). The equations formulated based on deuterium oxide dilution are fairly accurate at predicting whole-body fat ($r^2 > 0.70$), but not protein mass ($r^2 > 0.23$) in reproducing female swine. This method also requires venous catheterization for deuterium oxide infusion and blood collection. The equations formulated on the basis of body weight and backfat thickness are more accurate at predicting whole-body protein (r^2 range: 0.67 to 0.91) and fat mass (r^2 range: 0.80 to 0.95) in reproductive female pigs of various body sizes and stages of their cycle.

However, none of these techniques or derived equations distinguished between the size of the muscle protein mass and protein in the rest of the body. Modification of the comparative slaughter technique, by dissection of the carcass's primal cuts into muscle, fat and bone, allow a large proportion of the sow's muscle mass to be measured. Equations have been formulated to predict the muscle and fat mass of multiparous sows one week after weaning (Dourmad et al., 1996), and first-parity sows at parturition (Dourmad et al., 1998) from the dissected muscle and fat mass of the carcass and the non-invasive measures of live-weight and backfat depth. The estimate of muscle mass measured by these authors was, however, confounded by fat mass. These authors included intermuscular fat as a component of muscle mass, which increases the estimated value of muscle mass by 10% (Martin et al., 1981). They also included a primal cut (belly) that contains a large proportion (14%) of intermuscular fat, and in which the amount of intermuscular fat increases with animal fatness (Martin et al., 1981).

Because equations that solely estimate muscle protein mass in lactating sows are not available, the primary objective of the current experiment was to develop such equations from the muscle and fat mass dissected from the carcass, and non-invasive measures of body composition (live-weight and ultrasonic backfat measures). A wide range of body masses must be used to create such equations so that a young sow's muscle and protein mass may be estimated over the whole reproductive cycle (breeding, parturition, and weaning). To provide this wide range of body sizes, and to avoid slaughtering sows at parturition, we fed gilts in gestation and lactation to achieve highly divergent body masses at weaning (see Figure 6-1). This was achieved by feeding sows in gestation to either a standard or high body mass at parturition, and to lose a moderate or large amount of body protein in lactation. We also tested whether the accuracy of the formulated equations could be improved by the addition of other non-invasive measures of muscle mass such as muscle depth, area and composition.

The newly developed regression equations were used to estimate changes in the sow's muscle and fat mass over the course of gestation and lactation. Sow backfat thickness, and loin muscle thickness and area were measured ultrasonically, and body weight was measured during the course of the study. At weaning we measured the mass of a variety of tissues and organs, including bone, fat and muscle dissected from the primal cuts (shoulder, loin and ham). Muscle variables including protein, RNA and DNA and mRNA expression for protein in the main proteolytic pathway in muscle were also measured, and various aspects of the sow's lactational and reproductive performance were also assessed (Chapter 5).

6.2 MATERIALS AND METHODS

This experiment was conducted in accordance with the Canadian Council of Animal Care Guidelines, and was approved locally by the Institutional Animal Policy and Welfare Committee.

6.2.1 Experimental Treatments and Measurements

The experimental design, housing, and management of gilts during gestation and lactation are described in Chapter 5, and an outline of the treatments is described in Figure 6-1. In brief, the experiment was carried out in five replicates as a 2 x 2 factorial design with two gestational and two lactational feeding treatments. Genex gilts (Manor Hybrid x Large White or Manor Hybrid x Landrace; Genex Swine Group Inc.) were fed in gestation to achieve either a high or standard body mass at parturition. At parturition, gilts on the two gestational treatments had live-weights of 193 and 165 ± 1.8 kg and calculated whole-body protein masses of 30.0 and 24.3 ± 0.36 kg, but a similar backfat depth (~21.5mm). The sow's whole-body protein and fat masses were estimated using the equations of Whittemore and Yang (1989). In lactation the dietary treatments induced sows to lose a moderate or high amount of their parturition whole-body protein mass (approximately –11 and -17%), but to lose an amount of backfat that was moderate (-5mm) and that was not different among treatments. Twelve gilts were successfully allocated to each treatment, except only 11 were available for allocation to achieve a

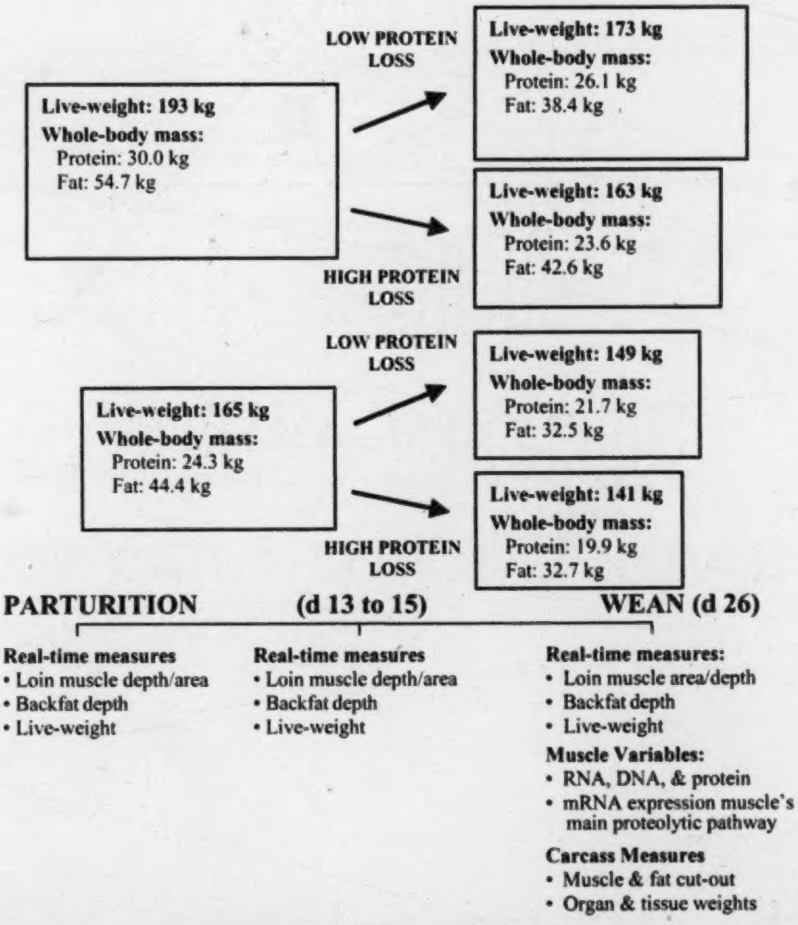

Figure 6-1 Diagram indicating the divergent live-weight and estimated whole-body protein and fat masses of sows at parturition and weaning. The sow's whole-body protein and fat masses were estimated using the equations of Whittemore and Yang (1989).

standard level of growth in gestation and to lose a high level of maternal protein in lactation. Progressive loss of body protein mass was associated with a decline in litter growth and ovarian function (index of reproductive performance).

Approximately every 16 d during gestation sows were weighed, and backfat depth, and loin muscle area and depth, were measured ultrasonically. These measures were repeated at the beginning of lactation (d 0 to 2), in mid-lactation (~ d 15) and at weaning (~ d 26). Within 2 to 3 h after weaning, sows were slaughtered and a muscle sample (2 to 4 g) was excised from the long head of the triceps brachii muscle, within a few minutes of slaughter from the left-hand side of the animal. The sample was immediately trimmed of connective and adipose tissue, frozen in liquid nitrogen, and stored at -70C until analyzed for RNA, DNA, protein, and mRNA by Northern hybridization analysis. The evening prior to slaughter feed was removed from the sows at 1800 to ensure that sows had been fasted for at least 16 h prior to slaughter.

The triceps brachii muscle is of mixed-fibre composition in the pig (Uhrin and Liptaj, 1992; McAllister et al., 1997), and is therefore representative of the majority of skeletal muscle in the body (Ariano et al., 1973; Maltin et al., 1989; Delp and Duan, 1996). The RNA:DNA ratio (capacity for protein synthesis) was used as an index of, and is highly correlated with, changes in the rate of muscle protein synthesis in conditions of nutritional deprivation (Millward et al., 1973, 1974; Goodman and Ruderman, 1980). The majority (~80%) of the cell's total RNA is composed of ribosomal RNA (cell's protein synthetic machinery) (Darnell et al., 1986). By dividing muscle total RNA concentration by an index of the tissue's cell number (DNA; Powell and Aberle, 1975) changes in the protein synthetic capacity/muscle cell unit may be assessed. The expression of mRNA in the main (ubiquitin-ATP-dependent proteasome) proteolytic pathway in muscle (Attaix et al., 1994, 1998) was used as an index of the rate of protein degradation. Increases in expression of muscle mRNA encoding proteins for this proteolytic pathway are associated with, and used as an index of, increased rates of protein degradation. These proteins include ubiquitin (Wing and Goldberg, 1993; Baracos et al., 1995; Medina et al., 1995), 14-kDa ubiquitin-conjugating enzyme (14-kDa E_2; Wing and Banville, 1994), and several subunits of the 20S proteosome (Baracos et al., 1995; Medina et al., 1995).

6.2.2 Carcass measures and dissection (cut-out) of primal cuts

The carcasses were sawn in half and chilled for 24 h at 4°C after removal of the head, front feet, kidneys, kidney fat, and viscera. The cold right half-side was cut into the primal cuts of the shoulder (picnic [lower shoulder], butt [upper shoulder], and hock), loin, ham, and belly. The shoulder, loin, and ham were separated into muscle, fat and bone, according to the boned-defatted cut-out procedure of Martin et al. (1981). It can be assumed that dissection (from cut-out) of the three primal cuts to their boneless, externally and internally defatted masses yields mainly muscle tissue and very little fat tissue. The weights of these respective tissues were recorded, and the body cavity, subcutaneous, and intermuscular fat depots for each cut were added together for the total fat in each cut. The total muscle mass of the sow at weaning was calculated as twice the muscle tissue in the half-carcass primal cuts (shoulder, loin and ham). These cuts contain the majority of the muscle tissue in the body. Other cuts that contain lean tissue, such as the belly, jowl, and ribs, were not separated into their various tissues because they are difficult and time-consuming to dissect. Total carcass fat was calculated as the kidney fat plus twice the fat tissue in the half-carcass lean cuts. The results of this dissection procedure were highly reproducible (Butterfield et al., 1962; Martin et al., 1981), because the procedure was performed by highly trained technologists at the Meat Research Section (Agriculture and Agri-Food Canada, Lacombe).

6.2.3 Ultra-sound measures

Sow backfat and loin muscle depth, and loin-eye area were measured using an Aloka SSD-210DXII Echo camera with a UST-5020 diagnostic real-time ultrasound (Aloka Co. Ltd., Tokyo, Japan) equipped with a 110mm wide 3.5 Mhz probe head (Overseas Monitor Corporation Ltd., Richmond, BC). The images were captured on video for later analysis of loin-eye area. To ensure proper acoustic coupling between the probe head and the back of the pig, a guide that approximated the curvature of the pig's back, was attached to the probe head (Animal Guides Fabrication, Lansing, NY). Scan Electrosound Gel (Electro Medical Services, Edmonton, AB) was placed between the probe head and guide and between the guide and the pig's back. The probe head, with attached guide, was then placed perpendicular to the medial plane of the pig's back to obtain a transverse view of the longissimus thoracis (loin-eye) muscle. This muscle is large, easily identified ultrasonically, and undergoes differential protein loss, as identified by changes in area, in sows exposed to various degrees of dietary protein deficiency (Touchette et al., 1998). All sites measured were located 50mm ventral and lateral to the midline of the pig. The loin-site was located 35mm anterior to the ileum, which in vivo approximates to being just forward of the pelvis bone. The mid-back site was aligned with the apex of the curvature of the last rib, and the grade-site was located 12 to 15 cm anterior to the mid-back site. The ultrasound images were captured from video using the Northern Exposure program. The images were rotated and adjusted for clarity using Adobe Photoshop 4.0 (Adobe Systems Inc.). The area around the loin-eye muscle at the mid-back and grade-site were measured using the Scion Image Beta 4.02 Win Program (Scion Corporation, Frederick, Maryland, 2170, USA). These areas were averaged and used in various analyses.

6.2.4. Analyses

Before analysis, individual muscle samples were pulverized in a mortar and pestle in liquid nitrogen and stored at -70°C until required for analysis.

i) Muscle RNA, DNA and protein analysis. The protein, RNA and DNA were separated and measured in the same sample using a modification of the Schmidt-Thannhauser procedure (Munro and Fleck, 1966). A detailed description of the methodology is provided in Chapter 4. In brief, 250 mg of frozen powdered muscle tissue was incubated for 10 min on ice with 4 mL of 2% (w/v) perchloric acid. The precipitate was centrifuged for 15 min at 2,800 g and 4°C, and washed. The pellet was re-suspended in 4 mL of 0.3N NaOH and incubated at 37°C for 1 h, the alkaline digest was cooled on ice, and 100 µL removed and diluted to make a 0.1N NaOH alkaline digest. This was later analyzed for protein using the micro-titre plate method of the BCA assay following the manufacturer's protocol (Pierce, Rockford, IL). Two milliliters of ice-cold 12% PCA was added to the remaining alkaline digest and incubated on ice for 10 min. The sample was centrifuged for 10 min at 2,800 g at 4°C, and the supernatant containing the muscle RNA was collected. The pellet was washed once in 2% PCA. The acid-soluble supernatant plus washings were diluted with distilled de-ionized H_2O to make a 1% PCA solution from which the muscle RNA was quantified spectrophotometrically using the formula of

Table 6-1 Half-carcass (right-side) cut-out of lean cuts into muscle and fat tissue in first-parity sows with a standard or high body mass at parturition that lost a moderate or high amount of protein during lactation

Parturition Mass	Standard Lean Mass		High Lean Mass		Significance [a]	
Lactation Loss	High	Moderate	High	Moderate	PBM	PL
Muscle tissue from primal lean cuts, kg						
Picnic	2.84 ± 0.12 [b]	3.23 ± 0.12	3.57 ± 0.13	3.77 ± 0.12	0.001	0.017
Hock	0.50 ± 0.03	0.54 ± 0.03	0.65 ± 0.030	0.62 ± 0.03	0.001	0.905
Butt	3.14 ± 0.12	3.39 ± 0.12	3.74 ± 0.13	4.00 ± 0.12	0.001	0.041
Loin	5.67 ± 0.29	6.58 ± 0.27	7.21 ± 0.30	8.08 ± 0.29	0.001	0.003
Ham	6.53 ± 0.25	7.31 ± 0.23	7.89 ± 0.26	8.49 ± 0.25	0.001	0.008
Fat tissue from all primal lean cuts, kg:						
Body cavity fat	0.27 ± 0.02	0.22 ± 0.02	0.26 ± 0.02	0.25 ± 0.02	0.615	0.144
Subcutaneous fat	5.28 ± 0.44	4.65 ± 0.42	6.45 ± 0.46	5.52 ± 0.44	0.029	0.079
Intermuscular fat	1.71 ± 0.11	1.56 ± 0.10	1.85 ± 0.11	1.67 ± 0.11	0.251	0.119
Other half-carcass cuts, kg:						
Belly	3.95 ± 0.26	4.05 ± 0.24	4.85 ± 0.27	4.87 ± 0.26	0.003	0.822
Ribs	1.84 ± 0.09	1.93 ± 0.09	2.12 ± 0.10	2.30 ± 0.09	0.002	0.148
Whole-carcass tissue mass from cut-out, kg						
Muscle mass [c]	37.3 ± 1.4	42.1 ± 1.3	46.1 ± 1.5	49.9 ± 1.4	0.001	0.003
Fat mass [d]	14.5 ± 1.05	12.9 ± 0.99	17.1 ± 1.09	14.9 ± 1.05	0.036	0.064
Kidney fat	1.90 ± 0.19	1.47 ± 0.18	2.02 ± 0.20	1.64 ± 0.19	0.474	0.036
Total carcass fat [e]	16.4 ± 1.2	14.3 ± 1.1	19.2 ± 1.2	16.5 ± 1.2	0.048	0.048

[a] Significance of the parturition body masses (PBM) and lactational protein losses (PL).
[b] Least-square mean ± standard error of the mean.
[c] Muscle mass (kg) = 2 x half carcass muscle tissue in primal cuts (shoulder, loin and ham).
[d] Fat mass (kg) = 2 x half carcass fat tissue in the primal cuts (shoulder, loin and ham).
[e] Total carcass fat (kg) = kidney fat + fat mass

Ashford and Pain (1986): RNA (ug/mL) = $(32.9\ A_{260} - 6.11\ A_{232})$ x dilution factor. The remaining acid-insoluble precipitate was incubation in 10% PCA at 70°C for 1 h, according to Forsberg et al. (1991), to extract the muscle DNA. The DNA was quantified using the diphenylamine method (Burton, 1956; Giles and Myers, 1965).

ii) Muscle RNA isolation and northern hybridization analysis. Details of the conditions for northern blotting, preparation of the probes, and pre-hybridization, and hybridization are described in Chapter 4. The riboprobe for ubiquitin was kindly provided by Dr. J. Walker (University of Wisconsin-Madison). The cDNA probe for 14-kDa E_2 was kindly provided by Dr. S. S. Wing (McGill University), and that of the proteosome subunit C9 was kindly provided Dr. A. Kumatori (Kumatori et al., 1990). Total RNA was extracted

Table 6-2 Skeletal muscle variables at weaning in first-parity sows with a standard or high body mass at parturition that lost a moderate or high amount of protein during lactation

Parturition Mass	Standard Lean Mass		High Lean Mass		Significance[a]	
Lactation Loss	High	Moderate	High	Moderate	LBM	PL
RNA:DNA	2.16 ± 0.09[b]	2.64 ± 0.09	2.54 ± 0.09	2.72 ± 0.09	0.015	0.001
Protein:DNA	0.55 ± 0.026	0.68 ± 0.026	0.67 ± 0.028	0.73 ± 0.027	0.003	0.001
RNA, ng/mg	483 ± 11	517 ± 11	492 ± 12	506 ± 11	0.924	0.040
DNA, ng/mg	231 ± 9	199 ± 9	194 ± 9	185 ± 9	0.007	0.020
Protein, µg/mg	122 ± 1[x]	131 ± 1[y]	129 ± 1[y]	129 ± 1[y]	0.059	0.002
RNA:protein	3.98 ± 0.10	3.97 ± 0.10	3.84 ± 0.10	3.88 ± 0.10	0.259	0.843
mRNA Expression						
Ubiquitin, 2.5kb	1.92 ± .17[b y]	0.95 ± 0.15[x]	1.18 ± 0.16[x]	0.87 ± 0.15[x]	0.015	0.001
Ubiquitin,1.2 kb	0.46 ± 0.05[y]	0.16 ± 0.05[x]	0.24 ± 0.05[x]	0.13 ± 0.05[x]	0.012	0.001
14-kDa E_2, 1.8 kb	1.38 ± 0.15	1.11 ± 0.12	1.34 ± 0.13	0.93 ± 0.12	0.418	0.008
14-kDa E_2, 1.2 kb	1.46 ± 0.20	0.70 ± 0.17	0.66 ± 0.18	0.52 ± 0.20	0.010	0.012
C9, 1.3 kb	9.99 ± 0.89[y]	10.8 ± 0.81[y]	10.5 ± 0.86[y]	6.0 ± 0.82[x]	0.017	0.033

[a] Significance of the divergent lean body masses (LBM) and lactational protein losses (PL).

[b] Least-square mean ± standard error of the mean

[xy] Within a row, means without a common superscript letter differ ($P < 0.01$).

from about 200 to 300 mg of muscle tissue using TRIzol™ (Gibco), according to the manufacturer's protocol (Chomczynski, 1993). Assessment of the purity and quantitation of RNA were determined by measures of absorbance at 260 and 280 nm. Ethidium bromide (1.7 µg) was added to each sample in the sample buffer, and samples were made up to the same volume with the addition of RNase-free distilled deionized H_2O prior to loading onto a 1.0 or 1.2% agarose-formaldehyde gels (Sambrook et al., 1989). All blots were exposed to X-ray film (Kodak) for varying amounts of time, dependent on the probe, and evaluated quantitatively using a Model GS-670 Imaging Densitometer (Bio Rad). The densitometric (Bio Rad) scans were normalized to the fluorescence level of the ethidium bromide stain of the 18S ribosomal band.

iii) Statistical analyses. All computations were performed using the GLM procedures of SAS (1990). Regression equations to prediction lean and fat mass of the animal were made using forward stepwise regression. These equations were based on the dissectable (from cut-out) muscle and fat mass of the carcass, and the live-weight, and average loin-eye area, loin muscle depth, backfat depth, and various muscle composition parameters at weaning. Effects of gestation feeding treatment on changes in calculated sow lean and fat

Table 6-3 Correlations between dissected muscle mass, calculated whole-body protein mass and indices of muscle mass at weaning

				Loin Muscle		
	Carcass muscle, kg	**Calculated whole-body protein [a], kg**	**Live-weight, kg**	**Mass, kg**	**Area, cm^2** (two sites)	**Depth, mm** (all sites)
Carcass muscle mass, kg	1.000	0.891 ***	0.846 ***	0.970 ***	0.620 ***	0.562 ***
Calculated whole-body protein mass [a], kg		1.000	0.961 ***	0.875 ***	0.522 ***	0.493 ***
Live-weight, kg			1.000	0.817 ***	0.538 ***	0.642 ***
Loin muscle mass, kg				1.000	0.606 ***	0.518 ***
Loin muscle area, cm^2 (mid-back & grade site)					1.000	0.647 ***
Loin muscle depth, mm (all three sites)						1.000

[a] Whole-body protein mass, calculated from Whittemore and Yang's (1989) equations.
[b] Significance of the correlations were *** = $P < 0.001$.

mass over gestation were assessed by repeated measures ANOVA. The model used was Y = Rep + BM + Br + BM x Br. Where Y = measured response; BM = gilt's body mass at parturition (standard or high); Rep = number of replicates in the experiment ($n = 5$); and Br = breed of the gilt's sire (Large White or Landrace). The effect of the lactation treatment on sow lean and fat changes over lactation were assessed by repeated measures ANOVA. The model used was Y = Rep + BM + PL + Br + BM x PL + BM x Br + PL x Br + PL x BM x Br. Where PL = lactation protein loss (high or moderate), and T = Time. For analysis of mRNA expression of various elements of proteolytic systems in muscle, membrane was included in the model. In the event of a significant T x BM or T x PL interaction, differences among Time within each treatment were computed using pre-defined orthogonal contrasts. The muscle parameters were assessed by ANOVA using the latter model. If significant treatment differences were detected ($P < .05$), these differences were computed using Fisher's protected least significant difference test. Correlation and simple regression equations were conducted between various ovarian and muscle measures, and sow live-weight, lean, fat, loin muscle parameters and backfat depth.

6.3 RESULTS

6.3.1 Carcass and muscle variables determined at weaning by treatments

There was a significant effect of the gestation treatment evident for all of the measures of muscle tissue, the carcass cuts of the belly and ribs and in the mass of subcutaneous fat (Table 6-1). These variables were all about 20% heavier ($P < 0.05$) in the larger sows indicating that these animals were supporting a larger mass of muscle and fat tissue. No difference in kidney fat was observed among gestation treatments. The total muscle mass of the sow and the muscle mass of all the cuts, except the hock, were also affected by the lactation treatment (Table 6-1). All the muscle groups measured at weaning were between 7 and 12% lighter ($P < 0.05$) in sows that lost the most protein in lactation, with the exception of the hock that did not differ in weight between treatments. The mass of fat tissue associated with the three primal cuts (shoulder, ham and loin) as well as the belly and ribs were also unaffected by the lactation treatment. There were no interactions among treatments for any of these variables.

i) Muscle variables at weaning. Again there were significant effects of both gestation and lactation treatments on the various muscle variables measured at weaning. The lactation treatment had the greatest impact on the muscle variables measured (Table 6-2). The RNA:DNA and protein:DNA ratios were lower and the DNA concentrations, and mRNA expression of both ubiquitin transcripts, and 1.2 kb 14-kDa ubiquitin conjugating enzyme transcript and C9 were higher in muscle from sows that had a small body mass at parturition, and also in sows that lost the most body protein in lactation ($P < 0.05$). There was an interaction of muscle protein concentration and mRNA expression of both ubiquitin transcripts and the proteasome subunit C9 among treatments. The most catabolic small sows had a lower (-6%) protein concentration and more than double the mRNA expression of both ubiquitin transcripts compared to the other three treatments ($P < 0.01$). The least catabolic large sows had a lower (-40%) mRNA expression of the proteasome subunit C9 compared to the other three treatments ($P < 0.01$).

6.3.2 Development of the regression equations

We initially examined the relationships amongst the variables determined at weaning, to determine the correlation between the muscle determined by dissection (from cut-out) of

Table 6-4 Correlations between muscle mass, calculated whole-body protein mass and biochemical muscle variables at weaning

	Muscle mRNA expression					Other muscle variables		
	E2 (1.8kb)	E2 (1.2 kb)	Ub (2.5 kb)	Ub (1.2 kb)	C9	DNA ng/mg	RNA: DNA	Protein: DNA
Carcass muscle mass, kg	-0.206 NS [a]	-0.458 **	-0.255 ‡	-0.335 *	-0.260 NS	-0.598 ***	0.540 ***	0.645 ***
Loin muscle mass, kg	-0.220 NS	-0.478 **	-0.300 *	-0.336 *	-0.298 ‡	-0.571 ***	0.530 ***	0.628 ***
Live-weight, kg	-0.211 NS	-0.532 ***	-0.148 NS	-0.211 NS	-0.402 **	-0.598 ***	0.482 ***	0.606 ***
Loin muscle area, cm^2	-0.229 NS	-0.284 ‡	-0.219 NS	-0.250 NS	-0.036 NS	-0.452 **	0.457 **	0.520 ***
Loin muscle depth, mm	0.001 NS	-0.398 **	-0.141 NS	-0.021 NS	-0.080 NS	-0.364 *	0.287 ‡	0.463 ***

[a] Significance of the correlations were: NS = non-significant; ‡ = $P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

the carcass and the indirect indices of these values. At weaning the muscle mass of the whole carcass and that of the loin were closely related ($r > 0.97$; $P < 0.001$) indicating that loin behaved in a manner reflective of the musculature as a whole (Table 6-3). Whole-body and loin muscle mass were strongly related to live-weight and to the whole-body protein mass calculated according to the regression equations of Whittemore & Yang (1989) which are derived from live weight and backfat thickness ($r > 0.84$; $P < 0.001$). Muscle depth and area determined by ultrasound correlated with the muscle mass of the loin and of the whole body ($r = 0.52$ to 0.62; $P < 0.001$) (Table 6-3). Of the biochemical variables measured in skeletal muscle (Table 6-4), the protein:DNA ratio was the most highly correlated with muscle mass and loin muscle mass and area ($r > 0.52$; $P < 0.001$). Muscle DNA concentration ($r > -0.45$; $P < 0.01$) and RNA:DNA ratio ($r > 0.45$; $P < 0.001$) were also related to muscle mass and area but to a lesser degree than protein:DNA ratio, and the mRNA expression was weakly or not significantly related (Table 6-4).

Prediction equations of whole-body muscle mass were formulated using the sow's live-weight (BW, kg) and backfat depth (BF, mm; average of all three sites) (Eq. [1]; $r^2 = 0.827$). When additional components were added to Eq. [1], these significantly ($P < 0.001$) improved the prediction equations: muscle protein:DNA ratio ($r^2 = 0.835$), litter growth rate (LGR, kg/d; $r^2 = 0.839$), loin-eye area (LEA, cm^2, Eq. [2]; $r^2 = 0.857$), and loin muscle depth (LD, mm Eq. [3]; $r^2 = 0.869$) The strongest estimate of muscle mass at weaning was based on an equation formulated using live-weight, loin muscle depth, backfat depth, and litter growth rate (Eq. [4]; $r^2 = 0.876$). However, this equation is only appropriate for estimation of muscle mass in lactation. Therefore Eq. [3] was used to estimate changes in the sow's muscle mass during gestation and lactation (see below; Table 6.8 and Figure 6-2).

$$\text{Muscle (kg)} = -4.84\ (\pm 4.01) + 0.394\ (\pm 0.027)\ BW - 0.633\ (\pm 0.123)\ BF \qquad [1]$$

$$\text{Muscle (kg)} = -11.50\ (\pm 4.33) + 0.352\ (\pm 0.029)\ BW + 0.433\ (\pm 0.147)\ LEA - 0.648\ (\pm 0.113)\ BF \qquad [2]$$

$$\text{Muscle (kg)} = -2.23\ (\pm 3.66) + 0.303\ (\pm 0.031)\ BW + 0.494\ (\pm 0.123)\ LD - 0.959\ (\pm 0.127)\ BF \qquad [3]$$

$$\text{Muscle (kg)} = 3.18\ (\pm 4.70) + 0.313\ (\pm 0.033)\ BW + 0.436\ (\pm 0.135)\ LD - 0.963\ (\pm 0.129)\ BF - 2.32\ (\pm 1.30)\ LGR \qquad [4]$$

The mass of fat cut out from the shoulder, loin and ham at weaning was strongly related to backfat thickness ($r > 0.81$; $P < 0.001$) and to the whole-body fat mass calculated according to the regression equations of Whittemore & Yang (1989) ($r > 0.84$; $P < 0.001$) (Table 6-5). Live-weight at weaning was also correlated with measured fat mass ($r > 0.50$; $P < 0.001$). Measuring backfat thickness at all three sites slightly improved these relationships compared to when depth was only measured at a single (mid-back) site. Equations to predict the fat mass were formulated using live-weight and backfat depth (Eq. [5]; $r^2 = 0.790$; $P < 0.001$). Addition of loin-eye area and muscle depth to Eq. [5] did not improve upon the prediction equation, but addition of muscle protein:DNA ratio did significantly improve the accuracy of the equation ($r^2 = 0.817$; $P < 0.001$). The best estimate of carcass fat mass was attained when live-weight, backfat depth, and lactation crude protein (CP, g/d) and total lysine (Lys, g/d) intakes were included in the equation (Eq [6]; $r^2 = 0.831$). However, this equation is only appropriate when estimating fat mass during lactation. Therefore Eq. [5] was used to estimate changes in the sow's fat mass during gestation and lactation.

Table 6-5 Correlations between fat mass, calculated whole-body fat mass and indices of body fatness at weaning

	Carcass fat, kg	Calculated whole-body fat mass[a], kg	Live-weight, kg	Backfat, mm all three sites	Backfat, mm mid-back site
Carcass fat mass, kg	1.000	0.844 *** [b]	0.512 ***	0.811 ***	0.767 ***
Calculated whole-body fat mass [a], kg		1.000	0.634 ***	0.896 ***	0.892 ***
Live-weight, kg			1.000	0.287 *	0.216 NS
Backfat , mm (all three sites)				1.000	0.964 ***
Backfat, mm (mid-back site)					1.000

[a] Whole-body fat mass calculated from the equations of Whittemore and Yang's (1989).

[b] Significance of the correlations: NS = non-significant; * = $P < 0.05$; *** = $P < 0.001$.

$$\text{Fat (kg)} = -7.75\ (\pm 2.69) + 0.078\ (\pm 0.018)\ \text{BW} + 0.762\ (\pm 0.074)\ \text{BF} \quad [5]$$

$$\text{Fat (kg)} = -10.64\ (\pm 2.93) + 0.098\ (\pm 0.017)\ \text{BW} + 0.839\ (\pm 0.078)\ \text{BF} + 0.013\ (\pm 0.006)\ \text{CP} - 0.309\ (\pm 0.117)\ \text{Lys} \quad [6]$$

6.3.3 Estimation of sow's body composition changes during gestation and lactation

The muscle and fat mass cut-out from the primal cuts was derived from sows that had a wide range of live-weights, backfat depths and loin muscle depths and areas at weaning (Table 6-6). These values were used to formulate equations to estimate the sow's muscle (Eq. [3]) and fat (Eq. [5]) mass. It is appropriate to use these equations to estimate changes in the sow's muscle and fat mass over the reproductive cycle (breeding, parturition, and weaning). This is partly because the range of the sow's live-weight, backfat depth and loin muscle depth and area at breeding and parturition were similar to those at weaning (Table 6-6).

At the end of gestation (parturition) the estimates of body muscle and fat mass were larger in the larger sows, but their relative fatness (lean to fat ratio) was similar (Table 6-7). The equations for whole-body protein and fat gain (Whittemore and Yang, 1989) and those formulated in this experiment to calculate muscle and fat mass in the carcass,

Table 6-6 Estimated and determined variables at breeding, parturition and weaning in first parity sows

	Mean	Std Dev	Minimum	Maximum
Breeding:				
Live weight, kg	128	16.0	95	162
Backfat[a], mm	18	4.52	10.3	30.0
Loin muscle depth[a], mm	38.3	5.49	30.0	54.0
Loin muscle area[b], cm^2	34.8	3.2	28.8	44.5
Parturition:				
Live weight, kg	181	20	150	215
Backfat[a], mm	21	4.79	13	35
Loin muscle depth[a], mm	41.1	5.90	28.0	52.7
Loin muscle area[b], cm^2	41.7	3.4	35.2	50.9
Weaning:				
Live-weight, kg	152	17.9	115	184
Backfat[a], mm	16.3	4.07	8.3	31.7
Loin muscle depth, mm	33.8	5.26	22.7	45.3
Loin-eye area[b], cm^2	30.9	3.5	23.3	40.5
Muscle mass, kg	44.4	7.54	29.5	62.6
Fat mass, kg	16.6	4.34	8.3	29.4

[a] Mean of the backfat and loin muscle depth measures taken at all three sites.
[b] Mean of the loin-eye area taken at two sites.

indicated similar relative gains of fat and protein-containing tissue during gestation. For example, the Whittemore and Yang equations predicted that the whole-body protein and fat mass were about 23% larger in the heavier sows at the end of gestation. Similar estimates were obtained using our equations, which predicted that the muscle mass was about 17% larger and the fat mass about 21% larger in the heavier sows ($P < 0.001$) (Table 6-7). However, the determinations of muscle depth and loin area using ultrasound lacked sensitivity in detecting changes in body composition. Loin muscle area was not different between the standard and heavier sows, and the loin muscle depth, while affected by treatment ($P < 0.05$), underestimated the magnitude of the difference determined directly (Figure 6-2). We did not slaughter animals at parturition and hence do not have any direct determination of body composition at that time. However, body composition data obtained at weaning showed a significant residual effect of the gestational treatment (Table 6-7). The magnitude of the differences attributable to the gestation feeding treatments in muscle (+ 21%) and fat mass (+17%) was quantitatively

Table 6-7 Changes in live-weight and body composition attributable to gestation feeding treatments, in first-parity sows fed to achieve a standard or high body mass at parturition. The effects of the lactation treatment are pooled within each parturition body mass treatment.

	Body Mass at Parturition [a]		**%**	
	High	**Standard**	**difference**[b]	**P**
AT PARTURITION				
Live-weight, kg	193 ± 1.9[c]	165 ± 1.7	+17.0	0.001
Loin muscle area, cm^2	40.1 ± 0.62	38.6 ± 0.56		0.092
Loin muscle depth, mm	43.5 ± 1.2	38.5 ± 1.1	+13.0	0.004
Backfat depth, mm	22.7 ± 1.0	20.0 ± 0.9		0.051
Estimated carcass muscle mass [d], kg	60.2 ± 0.78	51.4 ± 0.70	+17.1	0.001
Estimated whole-body protein mass [e], kg	30.0 ± 0.36	24.3 ± 0.35	+23.5	0.001
Estimated carcass fat mass [d], kg	24.8 ± 0.83	20.5 ± 0.74	+21.0	0.001
Estimated whole-body fat mass [e], kg	54.5 ± 1.68	44.4 ± 1.50	+22.7	0.001
Carcass muscle:fat ratio [d]	2.52 ± 0.10	2.57 ± 0.09		0.685
AT WEANING				
Half-carcass muscle mass, kg				
Picnic	3.67 ± 0.09	3.03 ± 0.08	+21.1	0.001
Hock	0.63 ± 0.02	0.52 ± 0.02	+21.2	0.001
Butt	3.87 ± 0.09	3.26 ± 0.08	+18.7	0.001
Loin	7.64 ± 0.22	6.13 ± 0.20	+24.6	0.001
Ham	8.19 ± 0.19	6.92 ± 0.17	+18.3	0.001
Total muscle (all carcass primal cuts)	24.0 ± 0.5	19.9 ± 0.5	+20.9	0.001
Half-carcass fat mass, kg				
Body cavity	0.26 ± 0.02	0.25 ± 0.01		0.615
Subcutaneous	5.99 ± 0.33	4.96 ± 0.30	+20.8	0.029
Intermuscular	1.76 ± 0.08	1.64 ± 0.07		0.251
Total fat (carcass primal cuts)	8.0 ± 0.4	6.9 ± 0.4	+16.8	0.036
Kidney fat	1.83 ± 0.15	1.69 ± 0.13		0.474
Carcass lean: fat ratio	2.89 ± 0.15	2.73 ± 0.14		0.448
Other half-carcass cuts, kg:				
Belly	4.86 ± 0.19	4.00 ± 0.18	+21.5	0.003
Ribs	2.21 ± 0.07	1.89 ± 0.06	+16.9	0.002

[a] The lactation treatments were pooled within each gestation treatment (parturition body mass).

[b] The % difference between high body mass and standard body mass sows.

[c] Least-square mean ± standard error of the mean.

[d] Muscle (Eq.[2]) and fat (Eq. [3]) mass from dissection (from cut-out) of the various tissues, calculated according to the sow's body weight (BW, kg), backfat depth (BF, mm) and loin-eye area (LEA, cm^2).

[e] Whole-body fat/protein mass calculated using the equations of Whittemore and Yang (1989), based on live-weight and backfat depth.

similar to those determined using both regression equations. Overall, the data obtained by dissecting the carcass strongly support the validity of the regression equations.

Table 6-8 Changes in the estimated muscle and fat mass of first-parity sows with a standard or high body mass at parturition that lost a moderate or high amount of protein during lactation

Parturition Mass	Standard Lean Mass		High Lean Mass		Significance[a]	
Lactation Loss	High	Moderate	High	Moderate	PBM	PL
At weaning, kg						
Muscle	35.5 ± 1.4[b]	42.7 ± 1.3	45.4 ± 1.5	50.8 ± 1.4	0.001	0.001
Fat	15.3 ± 1.0	15.0 ± 0.9	19.7 ± 1.0	17.3 ± 1.0	0.003	0.159
Loss in lactation, kg						
Muscle[c]	13.0 ± 0.81	9.4 ± 0.79	13.3 ± 0.83	10.2 ± 0.79	0.492	0.001
Muscle protein[d]	1.95 ± 0.10	1.21 ± 0.10	1.71 ± 0.10	1.31 ± 0.10	0.495	0.001
Fat[c]	5.2 ± 0.81	5.3 ± 0.76	6.1 ± 0.84	6.5 ± 0.81	0.214	0.762
Cumulative tissue loss[b] to d 15, % parturition mass						
Muscle[c]	13.8 ± 1.4	8.7 ± 1.3	13.0 ± 1.5	9.7 ± 1.4	0.939	0.004
Fat[c]	13.2 ± 2.1	11.8 ± 2.0	12.7 ± 2.2	15.0 ± 2.1	0.528	0.827
Cumulative tissue loss[b] in lactation, % parturition mass						
Muscle[c]	26.0 ± 1.6	18.0 ± 1.6	22.7 ± 1.7	16.6 ± 1.6	0.150	0.001
Muscle protein[d]	30.5 ± 1.6 ^{x}	17.9 ± 1.5 ^{z}	22.7 ± 1.6 ^{y}	16.6 ± 1.5 ^{z}	0.006	0.001
Fat[c]	24.3 ± 3.3	25.5 ± 3.1	23.4 ± 3.4	25.9 ± 2.2	0.932	0.558

[a] Significance of the parturition body masses (PBM) and lactational protein losses (PL).

[b] Least-square mean ± standard error of the mean.

[c] Muscle (Eq.[2]) and fat (Eq. [3]) mass calculated from the equations obtained for the prediction of cut-out muscle and fat tissue.

[d] Change in muscle protein during lactation was estimated using the estimated muscle mass (using Eq. [3]) multiplied muscle protein concentration. At parturition muscle mass was estimated to be 0.30 g protein/ g muscle wet weight, and at the weaning it was based on the concentrations in Table 6-2. For standard mass sows that lost the most protein, and the other three treatments these values were respectively 0.23 and 0.30 g protein /g muscle wet weight.

[xyz] Within a row, means without a common superscript letter differ ($P < 0.05$).

During lactation the regression equations were used to estimate the decrease in muscle and fat mass (Table 6-8 and Figure 6-3). There was a discrepancy between the results of the estimated loss of fat lost during lactation (Figure 6-3a), and the values measured directly at weaning (Table 6-1). Direct measurement of the sow's fat mass at weaning reveals that sows that lost the most protein had ~13% lower ($P < 0.05$) fat mass at this time. The majority of this difference in fat mass was located in the kidney fat and subcutaneous fat layer. Our regression equation (Eq. [5]) however, failed to detect a difference in the loss of body fat mass during lactation (-26.8 ± 3.0% parturition fat mass) between treatments.

The loss of muscle mass in lactation was initially calculated using Eq. [3] (Table 6-8 and Figure 6-3b). Since the protein content of muscle was reduced (-5.9%; $P < 0.002$) in the smaller sows on the protein restricted diet (Table 6-2), we calculated an adjusted muscle loss for these animals, taking into account the reduced content of protein per g of tissue. Muscle protein loss during lactation was affected by dietary treatment, and was greater in sows fed the lower protein diet (1.83 vs 1.26 kg; $P < 0.001$). When calculated as a percentage of the initial (parturition) muscle protein mass, the small sows fed the low protein lactation diet lost more muscle protein compared to the larger sows fed the low protein lactation diet (30.4 vs 22.7%; $P < 0.05$; Table 6-8). Sows fed the higher protein diet in lactation lost a similar (17.2%) amount of their parturition muscle protein mass independent of initial body size. There was good agreement between the estimates of muscle protein loss calculated by the regression equations and the muscle mass determined by cut out of the primal cuts at weaning. The change in muscle depth during lactation revealed decreases in muscle depth among treatments that were of similar magnitude to the losses of muscle protein mass (Figure 6-3c). A 17 and 23% decrease in parturition muscle depth was observed in sows that respectively lost the moderate and high amount of body proteins. Loin muscle area also decreased during lactation but the differences ($P < 0.01$) between treatments were not as pronounced (Figure 6-3d). Follicular fluid estradiol concentration increased as the percentage loss of muscle in

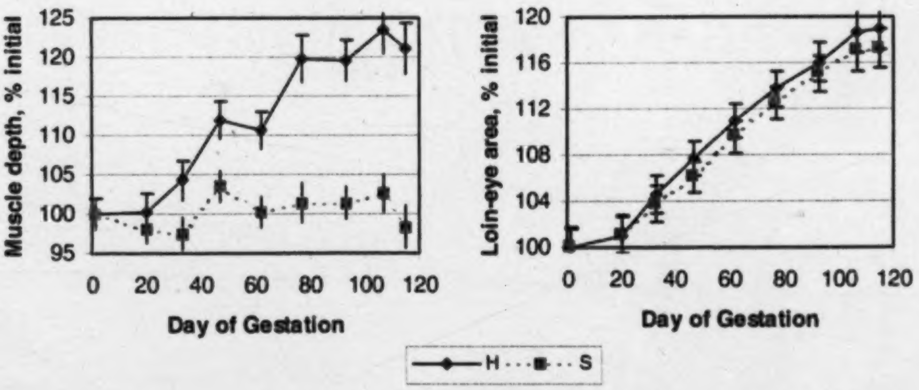

Figure 6-2 Percentage change in loin muscle area and depth in gilts fed to achieve either a standard (S) or high (H) body mass at parturition. Loin-eye area increased ($P < 0.001$) as gestation progressed, but a difference ($P < 0.05$) between gestation treatments was only observed for loin muscle depth.

lactation increased (r = 0.41; $P < 0.01$). However, no relationships were observed between fat loss in lactation and ovarian variables.

6.4 DISCUSSION

We achieved the primary objective of this experiment to formulate equations to estimate changes in the muscle mass of first-parity sows during lactation and gestation, based on non-invasive measures of body composition (live-weight, and ultrasonic measures of

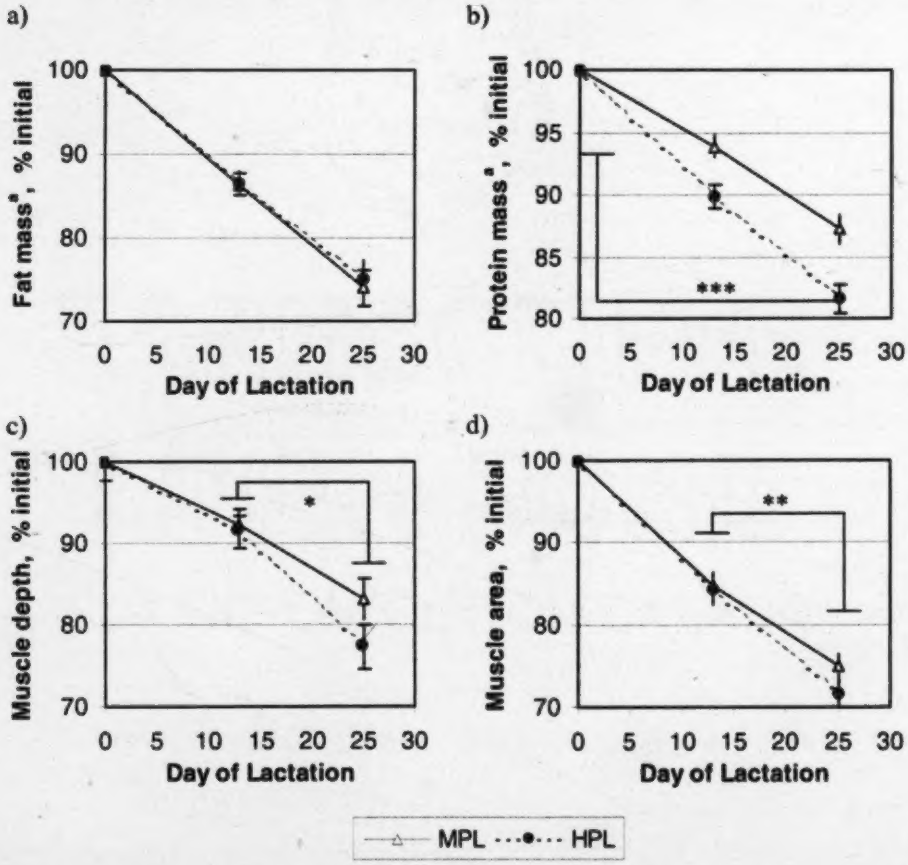

Figure 6-3 Change in estimated body a) muscle, and b) fat mass, and in c) loin muscle depth, and d) loin muscle area during lactation in first-parity sows that lost a moderate (MPL) or high (HPL) amount of protein. All values were calculated as a percentage of their initial (parturition) measure. Sow muscle and fat mass were calculated from Eq. [3] and [5], respectively. Symbols denote that the changes observed over the identified time periods differed, * = ($P < 0.05$), ** = ($P < 0.01$), *** = ($P < 0.001$) among treatments.

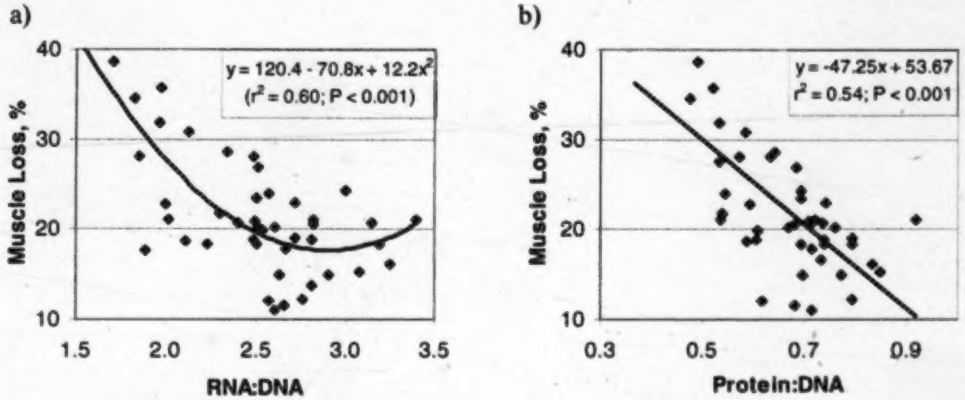

Figure 6-4 Regression of calculated muscle loss over lactation, as a percentage of muscle mass at parturition, against muscle a) RNA:DNA and b) protein:DNA ratio at weaning

backfat depth and loin muscle area and depth). The majority (~75%) of the skeletal muscle mass in the body was measured using the dissection technique of Martin et al. (1981). It can be reasonably assumed that the muscle mass in the remainder of the animal's body (belly, ribs, jowls etc.) changes in the same manner as that in the shoulder, loin and ham. The weight of individually dissected muscles (r = 0.80 to 0.99), or anatomically defined groups of muscles (r > 0.95), is highly correlated with total muscle mass (McMeekan, 1941; Cole et al., 1960; Orme et al., 1960; Butterfield, 1962). These relationships did not differ for steers over a wide range of ages (6 to > 30 months), weights, and breed types (Butterfield, 1962). All the muscle groups we measured in weaned sows differed by gestation treatment in a similar manner to one another. Thus, it is feasible to accurately predict the muscle mass of a sow by the weight of an anatomically defined group of muscles, over a wide range of weights, ages and genotypes. These equations can therefore be used to estimate the young sow's muscle mass over her reproductive cycle, because the range of variables included in the equation (live-weight, backfat depth etc.), measured at weaning, were similar to those measured at breeding and parturition.

The regression equations formulated in this experiment to estimate muscle mass (Eq. [1] to [4]) accurately predicted the sow's muscle mass, and explained between 83 and 88% of the total variance observed. It can be assumed that mobilization of protein from the muscle groups measured in this experiment was reflected in a similar degree of mobilization in the muscle tissue not measured. The majority of muscle in the body is of mixed-fibre type (Ariano et al., 1973; Maltin et al., 1989), and this muscle type is the main source of mobilizable protein in the body (Baille and Garlick, 1991; Fang et al., 1998). Also, the majority (97%) of muscle tissue measured in this experiment varied in a similar manner with lactation treatment; muscle tissue in most muscle groups was about 10% lighter in the most catabolic sows. The exception was the hock, a small muscle group in the shoulder, which did not show differential weight loss due to lactation treatment. This is probably because this tissue is a postural muscle in the pig and is protected from excess mobilization. Furthermore, a small decrease (-6%) in muscle protein composition was observed only upon mobilization of over 25% of the sow's muscle mass. These equations can therefore be used to estimate the percentage loss of muscle tissue mobilized from young sows. However if the degree of muscle protein mobilization is excessive, these equations will underestimate the degree of loss unless account is taken of the change in muscle protein content.

The inclusion of loin muscle area and depth into our equations to predict muscle mass only accounted for an additional 2 and 3.2% of the variation in the data, respectively, above that accounted for by live-weight and backfat depth. Loin muscle depth and area, and the amount of protein per muscle 'cell unit' (protein:DNA ratio) by themselves only accounted for 32, 38, and 42%, respectively, of the variation in the muscle mass of the weaned sow. In agreement, loin muscle area only accounted for a fairly small proportion (5 to 30%) of the variation in the muscle mass of the 95 kg pig (Kline and Hazel, 1955) and in a variety of ages, weights and breeds of cattle (Cole et al., 1960). Although loin muscle area did indicate changes in the muscle mass over time, it was not sensitive enough to measure the observed treatment differences in the muscle mass. Treatment differences were indicated by loin muscle depth. However, none of these measures by themselves is a good indicator of muscle mass. The muscle protein:DNA ratio did appear to be a better index of the percentage of muscle tissue mobilized, and accounted for 54% of the variability in this data.

The regression equations formulated to estimate the sow's fat mass (Eq. [5]) explained 79% of the total variance observed in this data set, and were therefore slightly less accurate than those formulated to estimate muscle mass. They were also slightly less accurate than equations formulated by others researchers who measured whole-body fat mass in sows by proximate analysis (Whittemore and Yang, 1989; Mullan and Williams, 1990; Everts and Dekker, 1995). In their equations, up to 95% of the variation in the data set was accounted for by live-weight and backfat depth. This may be partially explained by the fact that, in this experiment, we only measured half the weight of the estimated whole-body fat tissue. The discrepancy between the two estimates of body fat mass can be accounted for. In the experiments measuring whole-body fat mass, the fat mass of the whole carcass was measured. Whereas in our experiment, fat tissue was only recovered from the external (subcutaneous and body cavity) and internal depots (intermuscular) of three of the four primal cuts, and the fat surrounding the kidneys. Fat tissue was not recovered from the belly, a primal cut in which the intermuscular fat depot can account for about 14% of the tissue's weight (Martin et al., 1981). The intermuscular fat content of this tissue also varies with the fatness of the animal (Martin et al., 1981). The fat content of the hide, that associated with the mesentery, surrounding the mammary gland, and other organs, tissues and cuts (e.g. the jowls), and the tissue trimmed from the carcass cuts were also not accounted for in our measurement of carcass fat. This probably accounts for the slightly lower accuracy of our equations that estimate fat mass. However, the weight of the fat tissue we recovered was likely to be representative of the whole-body fat mass, because we measured the subcutaneous fat mass in three of the four primal cuts, and about 70% of the fat tissue in the pig's body is located subcutaneously.

6.4.1 Muscle protein loss associated with reduced animal performance

We previously related the decline in reproductive and lactational performance during lactation with the loss of whole-body protein rather than fat mass (Chapter 3 and 5). Upon a cumulative loss of about 10 to 12% of the sow's parturition whole-body protein mass (critical protein loss), estimated according the Whittemore and Yang (1989), litter growth declined and a decline in ovarian function (reproductive performance) appeared to be initiated. In this experiment we estimated this level of protein loss to be equivalent to about 14 to 18% of the sow's parturition muscle mass. The lowest ovarian function, and also litter performance, was observed in sows that had lost about 30% of their muscle protein mass, and had a lower relative muscle mass as signified by a lower muscle to bone ratio (Chapter 5).

Loss of more than half the muscle mass in the body is considered fatal (Newsholme and Leech, 1983). In our sows, this would amount to a respective loss of 34 and 40 kg of muscle mass, in the large and standard sows, before a risk of loss of life. This amount of muscle protein loss has been termed the body's mobilizable or labile protein reserve (Allison and Wannemacher, 1965; Swick and Benevenga, 1977). A decline in animal performance was first observed upon loss of 28 to 36% of the sow's labile muscle protein reserves, and the lowest animal performance was observed in animals that had lost about 60% of this amount. It is possible that upon loss of a certain proportion of the sow's muscle mass, metabolic warning signals are produced that reduce the energy and protein drain associated with milk production. They would also impair the reproductive axis to possibly delay the subsequent reproductive cycle and reduce the number of subsequent offspring. This would enable the sow to recover from the tremendous metabolic insult imposed on her in lactation and build up her body reserves. The muscle protein loss associated with initiation of these metabolic warning signals was estimated in these sows to be a loss of about 30% of the sow's labile muscle protein reserves. If the muscle protein loss in lactation is excessively large, the reproductive axis may shut down altogether, causing a cessation of reproductive cyclicity, presumably until a sufficient amount of the sow's muscle protein mass and other body reserves have been restored. This is likely the case for the sows that had lost about 60% of their labile muscle protein reserves.

6.4.2 Potential signals from muscle tissue that could inhibit the reproductive axis

Signals originating from the muscle mass, related to mechanisms that control muscle protein mobilization, could inhibit the reproductive axis. Different degrees of muscle protein loss are achieved by increasing the rate of muscle protein degradation and/or decreasing the rate of muscle protein synthesis. Muscle protein mobilization in lactation in the dairy goat and rat results from an increased rate of protein degradation (Baracos et al., 1991; Tesseraud et al., 1993; Pine et al., 1994ab) and a decreased muscle protein synthesis rate (Champredon et al. 1990; Pine et al 1994ab). However, the rate of muscle protein synthesis only declines in lactation if the degree of protein mobilization is large enough (Champredon et al., 1990; Baracos et al., 1991; Pine et al., 1994ab). Much less work exists regarding the mechanisms behind mobilization of muscle protein in the lactating pig. Higher fractional rates of muscle protein degradation were observed, throughout lactation, in first-parity sows fed low total lysine intakes (< 36 vs > 55 g/d) (Jones and Stahly 1999a, Yang et al., 2000). Similarly, we previously observed up-regulation of the main (ubiquitin-ATP-dependent proteasome) proteolytic pathway in muscle, at the level of gene expression, in first-parity sows that mobilized protein in lactation (Chapter 4). An increase in the extent of up-regulation of this pathway was observed as lactation progressed, and with the degree of dietary protein restriction. A larger decline in the capacity for protein synthesis in muscle was also observed in sows that mobilized more muscle protein in lactation (Chapter 4). This was reflected in the present experiment by a higher mRNA expression of genes in the main proteolytic pathway in muscle, and a lower capacity for protein synthesis in muscle at the end of lactation in the most catabolic sows that had mobilized the most muscle protein in lactation. Similarly, sows that had mobilized protein more extensively had a lower (-25%) capacity for protein synthesis in muscle at the end of lactation (d 26), compared to sows that lost 5% or less of their estimated protein mass (Chapter 2).

The degree of muscle protein mobilization could be communicated systemically by a muscle-derived factor whose secretion and blood level was proportional to muscle mass, or by either a diminished or imbalanced supply of amino acids appearing systemically. A negative signal would thereby be sent to the reproductive axis. This suggestion is supported by the negative linear relationship between the number for follicles on the ovary a weaning and mRNA expression of various genes in main proteolytic pathway in muscle. However, there does not appear to be a signal that causes conservation of muscle protein itself; the capacity for protein synthesis was lower, and the main proteolytic pathway in muscle continued to be up-regulated, in the most catabolic sows at the end of lactation. This suggests that the degree of muscle protein mobilization was still increasing despite the reduction in animal performance.

6.4.3 Conclusion

Equations were formulated to accurately estimate changes in the muscle ($r^2 = 0.87$) and fat ($r^2 > 0.79$) mass of first-parity sows in lactation, based on liveweight, backfat and loin muscle depth. These equations enabled the loss of muscle, associated with a decline in animal performance, to be measured; this was estimated to be a loss of 14 to 18% of the sow's parturient muscle mass. A continued loss of muscle mass, to 30% of the sow's parturient muscle, was associated with up-regulation of mRNA expression of the main proteolytic pathway and a reduction in the capacity for protein synthesis in muscle. This indicates that muscle tissue in animals that had lost over half their labile muscle protein reserve (60%) does not adapt to conserve its remaining protein reserves, as might be expected. The sow's reproductive performance (number of ovarian follicles at weaning) was negatively associated with mRNA expression of aspects of the main proteolytic pathway in muscle. However, a causal relationship between the amount of muscle protein mobilized and inhibition of the reproductive axis has not been established.

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CHAPTER SEVEN

GENERAL DISCUSSION AND CONCLUSION

7.1 CONCLUSIONS

A number of conclusions can be drawn from the experimental work conducted in this thesis that relate to 1) the amount of muscle protein mobilized by a first-parity sow in lactation, and 2) how this impacts on her litter growth and subsequent reproductive performance. The potential mechanisms that link body protein mobilization with reduced animal performance were also explored. The main conclusions were as follows:

1. First-parity sows prioritize additional nutrients (above their ad libitum intake) administered during lactation towards their maternal protein and fat reserves rather than the mammary gland and milk production. This suggests that growth and maintenance of the maternal body mass is a priority in young sows.

2. When dietary nitrogen is limiting during lactation sows mobilize their protein reserves and implement adaptive mechanisms to conserve this reserve. This is likely achieved in part by a reduction in the absolute rate of liver protein synthesis, and by decreased hepatic amino acid metabolism, reflected in reduced urinary nitrogen losses.

3. First-parity sows can sustain a loss during lactation of 10 to 12% of their previously existing (parturition) whole-body protein mass (14 to 18% of their parturition muscle mass) without any detriment to piglet growth or the determined indices of ovarian function. A larger body protein loss is associated with a progressive decline in litter growth, milk production and possibly milk protein concentration, and ovarian function. Our equations, based on the non-invasive measures of live-weigh, backfat and loin muscle depth, accurately estimated changes in the muscle and fat mass of first-parity sows in lactation.

4. Up-regulation of the ubiquitin-ATP-dependent proteasome proteolytic pathway (main proteolytic pathway) in muscle occurs at the level of gene expression in lactating sows that mobilize their protein reserves, and is evident throughout lactation. A decrease in the capacity for protein synthesis in muscle is also likely involved in the increased muscle protein mobilization in lactation. This suggests that in lactation muscle protein

mobilization is achieved by increasing the rate of protein degradation and decreasing the rate of protein synthesis. Changes in the degree of muscle protein mobilization are reflected in changes in these processes. A continued loss of 30% of the sow's parturition muscle mass was associated with further up-regulation of mRNA expression of the main proteolytic pathway, and a reduction in the capacity for protein synthesis in muscle. This suggests that the fractional rate of muscle protein mobilization continually increases throughout lactation under conditions of nutritional deficit, and that skeletal muscle does not reduce its rate of protein mobilization in lactation to conserve its remaining protein reserves.

5. Initially the concentration of most free amino acids in muscle increases in sows that mobilize protein in lactation. As lactation progresses the concentration of free essential amino acids decline and non-essential amino acids, most notable glutamine, remain elevated or increase further. The size of the change in amino acid concentration appeared to be related to the degree of muscle protein mobilized. Thus sows that lost the most protein in lactation were probably deficient in several essential amino acids (e.g. BCAA, phenylalanine, and threonine) and had an excess of some non-essential amino acids compared to their requirement for milk biosynthesis and mammary function.

6. Several factors likely contributed to the decline in milk production and milk protein synthesis in sows that had mobilized the most protein in lactation, and these included:

   i) a reduction in the availability, relative to the demand, for essential amino acids for milk biosynthesis. High peripheral non-essential amino acid concentrations may exacerbate this problem by inhibiting mammary gland essential amino acid uptake due to competition for use of mammary gland transporter systems.

   ii) the maximal rate of muscle protein mobilization was unable to provide all the amino acids required for mammary function and to maintain milk protein synthesis, because the maternal muscle protein mass was reduced to such a large extent in sows that had mobilized the most protein in lactation. A putative signal indicating the size of the muscle mass may be involved in regulating this process.

7. Independent of the animal's body size at parturition, first-parity sows that were more reliant on mobilization of reserves of protein in lactation expressed a poorer lactational and reproductive performance. The greater muscle protein reserve in large compared to small animals, likely serves to delay the onset of a decline in litter growth and ovarian function.

8. The signal(s) that cause impairment of the hypothalamic-pituitary-ovarian axis due to excessive protein loss in lactation may be related to the physiological changes that occur in the sow's body to promote maternal protein conservation. Changes in peripheral IGF-1 and insulin concentrations are not implicated, but changes in tissue sensitivity to these hormones cannot be discounted. Factors that could impair the reproductive axis include:

i) achievement of either 1) a smaller muscle mass, as indicated by a lower muscle to bone ratio and muscle protein concentration, and/or 2) large increases in the degree of muscle protein mobilization, could initiate warning signals that inhibit the reproductive axis and delay, or even prevent, the subsequent reproductive cycle.

ii) decreases in the availability of certain essential amino acids could 1) alter neurotransmitter concentrations centrally and thus directly inhibit the reproductive axis, and 2) alter follicular fluid amino acid concentrations and potentially inhibit the growth and development of ovarian follicles and the maturing oocyte.

7.2 DISCUSSION

The findings of this thesis do not refute the 'adipostat' (Frisch and McArthur; Frisch et al., 1980; Whittemore and Morgan, 1990) or 'metabolic' (Wade and Schneider, 1992; Wade et al., 1996) hypotheses. Rather they indicate that an additional factor is involved in the regulation of the reproductive axis. This is not surprising, because most complex functions have multiple regulators, so it would be unusual that only one mechanism regulates such an important physiological function as reproduction. We provided evidence that sows are able to maintain milk production and reproductive function by mobilizing their body protein reserves in lactation. However, progressive loss of body protein caused a reduction in both litter growth and ovarian function at weaning.

To avoid confounding our results with varying degrees of fat loss in lactation we elicited divergent degrees of body protein, but not fat, loss from our sows (Chapters 3 and 5). Subcutaneous fat is the main source of mobilizable fat in the pig, and changes in backfat depth provide the best index of changes in body fat mass in the pig. From the measures of backfat change it appears that we achieved our goal of creating animals that lost similar amounts of fat mass in lactation. This finding was supported by the measures of changes in internal fat (perirenal fat intermuscular fat). However, the potential effect of leptin (adipose tissue-derived hormone) cannot be discounted as a signal to the reproductive axis. Muscle tissue expresses both leptin mRNA and protein, but at 10-fold lower levels than adipose tissue (Wang et al., 1998), but it is not known how muscle or adipose leptin expression and secretion alters in conditions of restricted protein intake and muscle protein mobilization. It is also not known if leptin secretion and sensitivity changes with dietary protein deficiency. Further experimentation is required to determine the role of leptin in changes in reproductive functions during selective protein depletion.

Muscle is the main reserve of protein in the body, and depletion of this reserve can be catastrophic to the normal functioning of an individual, and fatal if more than 50% of body's muscle mass is mobilized (Newsholme and Leech, 1983). The size of the body's protein stores are closely regulated (Abbott et al., 1988), and it is possible that a factor exists, that is synthesized and secreted by muscle, and relates the size and changes in muscle mass centrally. This signal could impose changes in various physiological mechanisms to curb the continued maternal protein loss when critical levels of muscle loss are achieved, and ensure survival of the individual. We provided evidence that the fractional rate of muscle protein mobilization is not reduced in sows that have mobilized more than a critical amount of muscle protein in lactation (Chapter 4 and 6). The body likely targets expendable processes such as milk production, that cause the energy and protein drain from the body, by reducing milk protein synthesis and total milk production. It also targets reproductive processes, which although important for the future of the species, are unnecessary for the immediate survival of the individual and are a subsequent energy and protein drain on the body's resources. Some form of self-regulation may be in-built into muscle protein mobilization based on the existence of a maximal fractional rate of muscle mobilization. If an animal continued to lose muscle protein at the maximal fractional protein mobilization rate, the absolute rate of muscle protein mobilization would decline to reflect the decline in muscle mass. The following sections explore how excessive muscle protein loss might regulate animal performance.

7.2.1 *Could leptin be involved in regulating muscle and adipose tissue mass?*

Leptin has been suggested as the factor that enables the body to monitor its fat mass. Plasma leptin levels tend to increase in parallel with adipose tissue stores over the long-term, and are strongly correlated with body fat mass in normal fed individuals (Flier, 1997; Van Harmelen et al., 1998; Keisler et al., 1999). Leptin gene expression and in vitro secretion rates in the highly mobilizable adipose tissue depots (subcutaneous fat) in humans are more than double those in the less mobilizable adipose depots such as visceral or omental fat (Masuzaki et al., 1995; and Van Harmelen et al., 1998). Leptin gene and protein expression (Wang et al., 1998) and gene expression of the leptin receptor capable of signal transduction (Lin et al., 2000) have been detected in skeletal muscle. However, skeletal muscle leptin concentrations are 10-fold lower than those in adipose tissue, and leptin protein is also expressed at a much lower level in muscle compared to adipose tissue (Wang et al., 1998). It is not known in what degree muscle protein mobilization results in alterations in circulating leptin concentrations, compared with changes in fat reserves. It is similarly unknown what effects fat and protein mobilization have on circulating leptin concentrations in study models such as those used here. The effects of protein deficiency on leptin production by adipose tissue and muscle are also uncharacterized. The role of leptin in signaling changes related to muscle protein depletion and protein malnutrition thus remains to be determined.

7.2.2 How does a decrease in the sow's muscle mass impair the reproductive axis?

Nutrition-reproduction interactions have long been identified, and are probably related to changes in the availability of oxidizable metabolic substrates (Wade and Schneider, 1992;

Wade et al., 1996; Schneider et al., 2000). Nutrition-reproduction interactions have also been related to loss of body fat and changes in the degree of body fatness (Yang et al., 1989), and reduced energy (Reese et al., 1982, 1984; Nelssen et al., 1985; Johnston et al., 1989) and protein/lysine intakes (King, 1987; King and Martin, 1989; Jones and Stahly, 1999b; Yang et al., 2000ab). Reductions in muscle mass have also been related to impairment of the reproductive axis; King (1987) related body protein loss in lactation to the wean-to-estrus interval. By feeding lower lysine intakes in lactation, two authors also recently observed higher rates of myofibrillar protein breakdown in lactating sows that showed impaired reproductive function (Jones and Stahly, 1999ab; Yang et al., 2000b). These authors did not measure muscle protein loss, and did not associate a critical degree of protein loss with reduced reproductive performance.

Loss of muscle protein mass probably impinges on the reproductive axis by acting alone, or together with other factors such as the availability of oxidizable metabolic substrates, to inhibit reproductive function. The mechanisms by which alterations in muscle mass impact on the reproductive axis need to be elucidated. A few potential mechanisms are listed in the previous section (Section 7.1). One of these mechanisms is discussed in more detail below. Alterations in the skeletal muscle free amino acid pool likely reflect similar alterations in free amino acid availability in the peripheral system in catabolic conditions (Vinnars et al., 1975; Askanazi et al., 1980; Hammarqvist et al., 1989). If alterations in the skeletal muscle free amino acid pool, seen here, reflect changes in peripheral amino acid concentrations, then these changes may have an inhibitory effect on ovarian follicle and oocyte development and quality, and on the hypothalamo-pituitary axis. It is possible that the follicular fluid of sows that mobilized excessive amounts of muscle protein contained high levels of glutamine, and other non-essential amino acids, and lower levels of some essential amino acids. Free aspartate, glutamate, glutamine, glycine, alanine threonine, and methionine follicular fluid concentrations reflect plasma concentrations (Valazquez et al., 1977). Free histidine, phenylalanine and asparagine concentrations in the follicular fluid from human preovulatory follicles are higher than the levels observed in plasma (Jimena et al., 1993), and free cystine concentrations are lower than those observed in plasma (Valazquez et al., 1977). This could impact on oocyte development and maturation by reducing the uptake of essential amino acids due to competition for their uptake by various non-essential amino acids. It may also be conjectured that decreases in the concentration of certain essential amino acids in the presence of increased non-essential amino acids in the peripheral circulation could alter amino acid uptake centrally due to factors such as competition with amino acid transporters. As amino acids act directly or indirectly as precursors for neurotransmitters, then central neurotransmitter concentrations could be altered, which may inhibit the reproductive axis.

7.2.3 How does a decrease in the sow's muscle mass impair milk production?

Milk production in the pig and other species, assuming adequate growth and development of the mammary gland in gestation and an adequate supply of substrates and energy for milk production, is affected by the animal's genetic potential for milk production and the intensity and frequency of the suckling/milking stimulus. Milk protein production appears to be limited by the ability of the mammary gland to utilize amino acids taken up in addition to those required to maintain optimum milk protein biosynthesis and mammary function. Increasing (+23%) the intake of rumen-protected protein to lactating multiparous dairy cows increased (+26%) essential amino acid uptake by the mammary gland but only increased milk production and milk protein yield by 7%. The supply and mammary uptake of non-essential amino acids was unaffected by the increase in dietary treatment (Metcalf et al., 1996). Similarly, increasing (+26%) the essential amino acid supply to lactating first-parity dairy cows caused a small (+5%) and non-significant ($P < 0.10$) increase in milk protein output, despite the increased (+34%) mammary essential amino acid uptake (Metcalf et al., 1994). These results suggest that the mammary gland transport systems for essential amino acids are not saturated in normal lactation, and that there may be either net deposition of mammary proteins or oxidative loss of amino acids in the mammary under these conditions.

An imbalanced supply of essential amino acids reduces milk protein but increases milk fat output. An induced histidine deficiency in the lactating goat reduced milk protein output by about 20%, despite an approximately 33% increase in mammary blood flow (Bequette et al., 2000). Similarly in dairy cows, a deficiency in post-ruminal methionine, lysine or histidine supply reduced the yield of milk protein by 15%. But a deficiency in post-ruminal lysine and histidine increased the yield of milk fat by 35 and 44% respectively (Weekes and Cant, 2000). This supports our suggestion that the provision of an imbalanced amino acid mixture, such as that released upon muscle mobilization, could directly impair milk protein synthesis and mammary function. In contrast to the amino acids mentioned above, a post-ruminal deficiency in BCAA supply to the mammary gland had no effect on milk protein and fat yield in the lactating dairy cow (Weekes and Cant, 2000).

7.3 FUTURE RESEARCH DIRECTIONS

The conclusions of this thesis provides a basis to propose several areas of research and suggest that a number of questions be addressed, including the hypotheses listed below.

7.3.1 Proposed new set of hypotheses

1. Changes in the concentration of amino acids in the free amino acid pool, in sows that have mobilized a large proportion of their muscle protein mass, are reflected in peripheral amino acid concentrations.
2. The composition of amino acids released upon muscle protein mobilization from the sow does not match that required for milk protein synthesis and mammary function.
3. Supplementation of sows that have mobilized a large proportion of their muscle protein reserves in lactation with essential amino acids at the end of lactation will prevent the decline in milk production and ovarian function observed.
4. Essential amino acid mammary gland uptake is inhibited by the presence of an amino acid mixture that reflects the plasma concentrations during a state of protein mobilization. Glutamine probably plays a key role in this response.
5. Oocyte nuclear and cellular maturation is inhibited when oocytes are cultured in the presence of an amino acid mixture reflecting plasma concentrations during a state of protein mobilization.

7.3.2 Future experiments to test these hypotheses

1. Measurement of both plasma and muscle free amino acid concentrations throughout lactation in first-parity sows that mobilize a large or a small amount of protein during lactation.

2. Measurement of concentrations of, and arterio-venous differences in, amino acids across the hind-limb of the dry sow in the post-absorptive state (preliminary experiment), and in mid- and late lactation in sows fed to lose a small or large amount of body protein. To identify the relative amino acid deficiency and surfeit, these amino acid profiles will be compared with the mammary amino acid uptake of multiparous sows that lost a small amount of body tissue (9.0 kg liveweight loss) and maintained a modest (1.64 kg/d) litter growth rate over their 21 d lactation (Trottier et al., 1997).

3. In a 2x2 factorial experiment, feed sows 1) to lose a small of a large amount of their body protein reserves during lactation and 2) supplement sows with a mixture of essential amino acids, that are seen to be deficient in experiment two (above), from d 16 of a 21 d lactation. Measure litter growth, milk composition and ovarian function (reproductive performance).

4. Measure the arterio-venous difference in amino acid concentration across the mammary gland, over two days, in mid- and late-lactation in first-parity sows fed to lose a small or large amount of body protein. In both periods, supplement sows that lost a small amount of protein in lactation, on one day, with high levels of some non-essential amino acids, and test if this changes mammary gland amino acid uptake or milk protein composition.

5. Culture generic porcine oocytes in concentrations of amino acids that reflect peripheral amino acid concentrations in sows that have mobilized an excessive amount of muscle protein to test whether oocyte nuclear and cytoplasmic maturation is impaired.

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